Vol. 11 No. 3

PLANT PHYSIOLOGY

JULY, 1936

GENERAL NATURE OF THE PROCESS OF SALT ACCUMULATION BY ROOTS WITH DESCRIPTION OF EXPERIMENTAL METHODS[1]

D. R. HOAGLAND AND T. C. BROYER

(WITH NINE FIGURES)

Introduction

The discussion in this paper is based on the assumption that the reader is familiar with earlier work on *Valonia, Nitella,* and *Chara* cells (**3, 5, 15–17, 23–25, 42**), and with the series of recent reports by STEWARD (**32–36**) and his co-workers (**2, 37–40**) on accumulation of salts by storage tissues. These latter researches yielded clear evidence that salt accumulation (movement of cations and anions into the vacuole against concentration gradients) by storage tissues is dependent upon the metabolic activities of living cells, reflected in aerobic respiration. It was concluded that capacity for maintained salt accumulation by these tissues is associated with a state of intense cell metabolism characteristic of cell growth and cell division. The problem now to be discussed is that of salt accumulation by roots with particular reference to metabolic processes. This problem has special significance for students of soil and plant interrelations, and at the same time has broad ramifications in the field of general physiology. One of the most fundamental of cell functions is involved.

Materials and methods

Consistent and significant conclusions on salt absorption by root cells must rest on experiments in which control is exercised over certain variables, some of which have failed to receive adequate attention in previous investigations. For this reason, it is deemed advisable to describe in detail

[1] Preliminary reports of some of the most essential features of this investigation, conducted during the past several years, have been presented before the American Society of Plant Physiologists (1932–33).

the methods employed in the present investigation. Experiments were made on entire plants of various species and on excised root systems of the barley plant (*Hordeum vulgare*). This paper will be restricted primarily to the latter. Many of the fundamental questions of salt absorption by root cells can be studied most effectively by eliminating, during a brief experimental period, the complications of root and shoot relationships. The method was to produce for each experiment a large number of uniform absorbing root systems with certain desired characteristics resulting from the conditions imposed during the preliminary growth period, and then to study salt absorption by these systems over short periods. The nature of the process of salt accumulation was the objective, not the cumulative effects of nutritional factors on plant development and their secondary effects on salt absorption.

Root tissues in an appropriate nutritional and developmental state seem to form the most active salt-accumulating system so far investigated. It will be shown that root tissues of this kind under conditions favorable to active aerobic metabolism have the capacity to accumulate large amounts of K, Br, or NO_3 within a 10-hour period. In fact, significant salt accumulation by highly active root systems can occur within 2 hours. Young growing roots are especially adapted to the study of a number of general problems of salt accumulation by living plant cells. So far as the writers are aware, experiments on excised roots similar to those described in this paper have not been reported heretofore. PIRSCHLE and MENGDEHL (**29**), in their studies on excised root systems, did not provide the conditions required for active salt accumulation.

The "Sacramento" variety of barley was used in all experiments except those performed during the preliminary stages of the investigation. This detail is of some importance for the reason that this variety of barley is almost immune to mildew attack under our greenhouse conditions. With other varieties we have found serious difficulty in producing disease-free plants.

FACTORS ESSENTIAL TO DEVELOPMENT OF ROOT SYSTEMS

Our first attempts to induce an active accumulation of potassium salts by excised root systems were not very successful, even when suitable external conditions for salt absorption were provided. Usually there occurred some net accumulation of Br from dilute KBr solutions, but little or no net accumulation of K. Sometimes there was a loss of K from the root system, as a whole, during the experimental period. Without entering into the details of these experiments, we shall present in brief outline certain factors essential to the development of root systems which have a high

capacity for salt absorption, as they have been disclosed in this investigation.

AGE AND DEVELOPMENTAL STATE.—An excised root system as a whole will exhibit maximum potentialities for salt accumulation only when actively metabolizing cells predominate in the system. If they do not, the accumulation of salts by active cells may be obscured by the loss of salts from inactive or moribund cells, and entirely misleading conclusions reached regarding the relations of temperature, aeration, and other factors to salt accumulation. When grown in a favorable climatic and nutritional environment, barley plants 6 weeks or more old can furnish root systems suitable for the study of certain phases of the problem of salt accumulation and several experiments on root systems of this age will be discussed. In general, however, it is highly advantageous to employ very young root systems. If these are developed under properly selected cultural conditions they consistently provide tissues with a high capacity for salt absorption. In the standard technique of this investigation, plants were grown in a special culture solution for a period of approximately 3 weeks preceding the excision of the root systems.

NUTRITIONAL HISTORY OF ROOT TISSUES.—The capacity of root cells to accumulate salt during a brief experimental period is profoundly influenced by the nutritional status of the plant at the time the experiment is begun. It is, therefore, very important to control the concentration, composition, and volume (per unit number of plants) of the nutrient solution in which the plants are grown in preparation for absorption experiments. Healthy root tissues may be caused to vary extremely in their salt content. When the volume and concentration of the nutrient solution are sufficiently limited, the supplying power of the external solution does not keep pace with the translocation of salt from root to growing shoot, and, as a consequence, the root suffers partial depletion of its salt content. An initial low-salt condition is a requirement for the most rapid accumulation of salt by excised roots in a subsequent absorption period. It is, of course, necessary that the supply of nutrients shall not be so restricted during the preliminary growth period that root growth and metabolic activity become impaired through starvation effects. Low-salt root tissues developed under our experimental conditions also have a high sugar content, a point to be discussed presently.

SEASONAL EFFECTS ON DEVELOPMENT.—Plants were grown at all seasons of the year in the greenhouse, with a standardized technique, and the observation was made that yields of shoot varied comparatively little (being limited by nitrate supply) but the root systems grown during several winter months had much lower fresh weights than those of the best summer root

systems. The latter had an extensive development of fine lateral roots, which were decidedly less abundant on winter root systems. Further data must be acquired before positive general statements can be made concerning effects of seasonal conditions on capacity for salt accumulation per unit weight of tissue, but, on this basis, certain sets of roots grown during highly unfavorable seasons had a relatively low capacity for salt absorption.

The influence of season on root growth, under the given greenhouse conditions, must be ascribed rather to the intensity or quality of illumination than to temperature. During one season, the incidental observation was made that thorough cleansing of the glass of the greenhouse roof was followed by a large increase in the ratio of root to shoot. Extending the period of illumination with Mazda lights of considerable intensity had no significant influence on the weight and nature of the root system; neither did an increase of greenhouse temperature. Brief exposures to a mercury arc lamp caused slight injury and retardation of elongation in shoots, but produced no important modification of the root system. The proportion of root to shoot could not be materially increased in the winter season by the various modifications of the nutrient solutions tested. Some specific data on seasonal effects on root-shoot relations are presented elsewhere (**14**).

Culture technique

In earlier experiments, the containers for the culture solutions were glass jars of 2-liter capacity, of the type commonly employed in water culture experiments. Later, 20-liter earthenware jars or large 115-liter tanks made of pure iron served as culture vessels. The tanks and other iron containers were thoroughly coated with an asphalt paint, which has not shown the slightest toxicity to plants. In the experiments with young plants, which comprised the major part of the investigation, shallow trays of approximately 4-liter capacity were used successfully. Over the top of the tray[2] was placed a cover made of black iron, coated with asphalt paint. This cover was perforated with holes 5.7 cm. in diameter, for receiving corks, each of which was bored with 7 holes, in which the seedlings were fixed with non-absorbent cotton in the usual manner. Each cover accommodated 24 corks, so there were 168 plants in each set (fig. 1).

In some experiments use was made of coated iron trays with dimensions similar to those of the trays described above, but provided with broad flanges, the covers having corresponding flanges. By means of small bolts, the cover could be fastened tightly to the tray when it was desired to pass gases other than air through the solution, but in the majority of experiments 4-liter stoppered bottles were employed for this purpose.

[2] Granite-ware pans served the purpose adequately but pans with broken enamel should not be used. It may be desirable to coat the inside of the pan with asphalt paint.

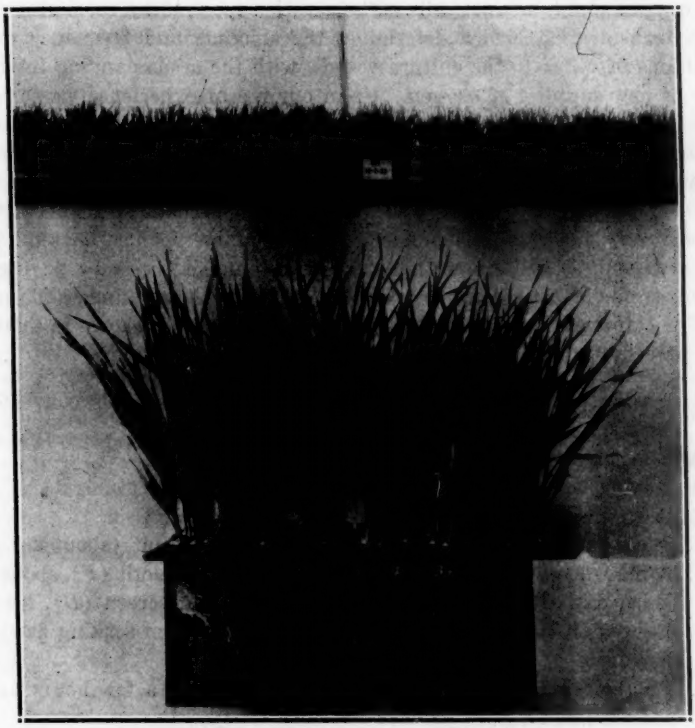

FIG. 1. Illustration of method of growing uniform sets of barley plants to obtain excised roots for absorption studies. 168 plants in each set.

Ordinarily the roots were not given forced aeration during the preliminary period of growth. The shallow trays with loosely fitting covers allowed sufficient exchange of gases to permit excellent root development. The type of root system can be modified by forced aeration, but the roots so aerated for a long period become partially depleted of sugar, and this may sometimes constitute a disadvantage for the later study of salt absorption. When plants are grown in stoppered jars or bottles, without passing a stream of air through the solution, lack of aeration may prevent the development of root systems highly adapted to absorption experiments, especially if the barley plants are grown under unfavorable seasonal conditions. The discussions of ZIMMERMAN (**43**), and of CANNON (**4**) regarding the movement of oxygen from shoot to root are of interest at this point. According to their evidence, conditions for photosynthesis would influence

the oxygen supply of root cells. The aeration received by roots also varies with transpiration, which determines the amounts and frequency of the additions of water to the culture vessels, with the accompanying introduction of new supplies of oxygen. From our recent experience we conclude that tanks, trays, or very large jars with loosely fitting covers are, in general, greatly preferable to smaller, stoppered bottles or jars, in growing plants in preparation for salt absorption studies, not only because they assure better aeration, but also because the mechanical manipulations become very laborious when many small containers must be separately handled; and, as will appear later, we have found it necessary during the course of our investigations to utilize an extremely large number of plants.

The equipment for the germination of barley seeds is shown in figure 2. The procedure for the germination of seeds was as follows:

1. Soak seed in tap water about 18 hours (overnight is convenient) at 25° C.
2. Assemble thoroughly cleaned equipment (fig. 2).
3. Arrange seed carefully upon the netting (*E*).
4. Adjust screen (*D*) and water-soaked cloth (*F*).
5. Place in the dark, in a warm constant environment (about 25° C.).
6. When shoots begin to pierce through the cheesecloth (*F*) about the third day after soaking, remove this cloth and screen (*D*), leaving the remaining assembly until the fourth day from soaking as specified in 5.
7. On the fourth day, transfer to direct light after a few hours under shaded light.
8. On approximately the seventh day, when shoots have attained a height of about 9 or 10 cm., the seedlings are carefully withdrawn, one by one, selected for uniform development, and fixed in the holes in the supporting corks with non-absorbent cotton.
9. The corks containing the seedlings are then set in place in the covers of the shallow trays described above.

For certain species of plants, germination in pure silica sand may be advantageous, the seedlings being transplanted to corks when size and vigor permit.

In the present investigation all seedlings were germinated in tap water (of very low salt content), limiting salt nutrition primarily to that furnished by the seed up to the time of the transplanting into the culture solution employed for the preliminary growth period.

Increasing the supply of oxygen by forcing air through sintered glass aerators placed in tray *B* increased slightly the percentage of germination,

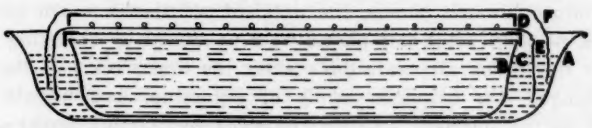

FIG. 2. Diagrammatic sketch of equipment for germination of barley seeds.

A. Royal enamelware oblong stove pan, 40.6 × 28 × 6.3 cm. containing tap water.

B. Pyrex utility or baking dish 32.1 × 21.5 × 5 cm. containing tap water or nutrient solution in which the seedling roots develop. (This tray should be maintained at nearly full capacity of water during the germination period.)

C. Double-tinned, brass wire screen, 8 meshes per inch (varying mesh with type of seed); height of metal side 1.2 cm.; length and width to permit screen to fit loosely over tray *F*.

D. Screen as under *C*, with length and width 0.6 cm. less than *C*.

E. Washed, single thickness of bleached mosquito netting, stretched over *C*, and dipping into water contained in *A*.

F. Washed, double thickness of bleached cheesecloth, stretched over *D* and dipping into water contained in *A*.

but this procedure has not been adopted in the experiments now reported. Aeration of this kind would modify, to some extent, the metabolic state of seedling roots.

NUTRIENT SOLUTION

The standard nutrient solution for the preliminary 3-week growth period had the following composition:

KNO_3	0.0025 M
$Ca(NO_3)_2$	0.0025 M
$MgSO_4$	0.001 M
KH_2PO_4	0.0005 M

For each set of 168 plants 3800 cc. of this solution were used. A solution of double this strength was substituted in some of the earlier experiments of longer preliminary growth periods. Also special low-potassium solutions were employed in certain instances.

Iron, as iron tartrate, 0.5 per cent. solution, was added to the nutrient solution in the proportion of 1 cc. per liter. Additions of the iron tartrate solution were repeated as necessary (usually twice each week) to prevent chlorosis. Impurities in the nutrient solution, and the supply of boron or other supplementary essential elements in the seed, made it unnecessary to introduce these elements during the brief period of growth; but this point requires attention in growing plants of certain other species. Possibly it will also become important in the study of special problems of metabolism in the barley root.

To produce low-salt plants, the nutrient solution was not changed in the 3-week period. High-salt plants were produced by changing the solution every day or on alternate days, after the first week. If the solution is not changed, the original supply of potassium and nitrate becomes exhausted in the nutrient solution, followed by partial depletion of the salt content of the root cells, through translocation of salt from root to shoot, as already explained.

Preparation of root systems for study

The root systems were excised just below the seed residue, and then thoroughly washed twice with distilled water. Afterward they were centrifuged by the technique described below, following which, sets of roots, each representing 168 plants, were immersed in the experimental solution, contained either in the shallow trays already described, or in 4-liter, wide-mouthed bottles provided with stoppers, through which tubes for aeration were passed. At the end of the absorption period, the roots were removed from the solutions, re-washed, and re-centrifuged. Fresh weights were recorded, and the roots were then placed in closed jars which were kept at −15° C. for 48 hours or more, according to convenience. The volumes of residual culture solutions were measured and these solutions kept for analysis when this was desired.

In a number of preliminary experiments with older root systems, attempts were made to obtain a uniform mass of root tissues by cutting root systems into short sections (2 to 5 cm.) and thoroughly mixing the segments in distilled water. Random samples of 75 to 125 gm., centrifuged fresh weight, were then withdrawn for the absorption experiments. This procedure yielded some useful results, but it soon became evident that it was desirable to employ for each treatment a very large number of entire root systems excised from young plants, in order to reduce errors of variability, to provide tissues of higher average metabolic activity, and to limit injury from cutting to a minimum. However, a later experiment showed no significant effect of cutting on respiration.

Aeration technique

The various devices employed for passing air or other gases through the solutions in which the roots were immersed during the absorption period were: manifolds of pure rubber tubing pierced with needle holes, block tin tubing similarly pierced, and Folin bulbs (for use with bottles). The most satisfactory aerators were those made of sintered glass, according to a technique described by Furnstal and Johnson (8). The small sintered glass aerators can be linked together in manifolds of diversified form and size suitable for jars or bottles, shallow trays, or large tanks. The

important point is that the solution shall be rapidly and uniformly aerated with very small gas bubbles. The air, supplied from a large compressor connected with a reservoir, was passed through water and filtered through cotton. Gases other than air were obtained from tanks under pressure, and in certain experiments the gas was specially purified. The rate of aeration was approximately 1.5 cu. ft. per hour for each set of roots. This rate was shown to be adequate, since it could be decreased considerably without affecting the results significantly.

The masses of root tissues were carefully separated so that air or other gases could readily reach all root surfaces. The roots normally remain suspended in the solution, and the stream of gas obviates the necessity for mechanical stirring. Stirring by the gas stream renews the salt solution at root surfaces and this doubtless is a secondary factor which influences the rate of salt absorption, but stirring alone without supplying oxygen is ineffective, as shown by experiments in which nitrogen gas, or gas mixtures very low in oxygen, were substituted for air.

Technique of centrifuging roots and of expressing sap

The root tissues were loosely tied in cheesecloth and submerged for a few minutes in distilled water. The cloth bags were then suspended (properly counterpoised) from the arms of a centrifuge head and rotated at the relatively slow speed of 400 r.p.m. for 5 minutes. This treatment caused no apparent injury and close reproductions of fresh weight could be obtained, within less than 1 per cent. variations. The moisture content of the centrifuged roots varied from 92 to 95 per cent. for sets grown at different times. It was altered very little during the absorption period, although slight increases occurred in certain experiments in which a large amount of salt was absorbed. With regard to osmotic relations, it is to be noted that while salt is being accumulated, sugar is being utilized.

The frozen root tissues were rapidly thawed in the closed jars in which they were originally placed and the sap quickly expressed in a heavy steel mechanical press by a reproducible technique (**6**). The sap so expressed was clear. The volume of sap recovered was nearly always equivalent to approximately 80 per cent. of the total moisture present in the tissues. Press cakes were dried at 80° C. and dry weights taken. Estimates of approximate total volumes of sap were calculated from these data when necessary. All determinations, except those for stable salts, were started within a few hours after expression of the sap. Sap retained for further study of salt content was preserved with a layer of toluene. This investigation is not concerned with those enzymatic changes of certain organic constituents which may occur in frozen tissue, or during freezing and thawing.

Clearly, expressed sap is a composite fluid derived from cells which belong to different tissues. Despite the differences in salt content which may prevail even in a single isolated root (PREVOT and STEWARD, **28**), or between roots at different developmental stages, this composite fluid represents a mean composition of sap upon which the salt accumulation by these accurately sampled root systems may be determined and compared. While the writers do not contend that the expressed sap is identical with the vacuolar sap as it existed in any particular living cells, they believe that in the light of present knowledge, the composition of expressed sap is an adequate basis for drawing conclusions concerning the general nature of the process of accumulation of mineral solutes by roots, with which they are alone concerned at present. The consistent relations which have emerged between salt accumulation and the different variables (both internal and external) are sufficient justification for this procedure.

When the amounts of K and Br withdrawn from solution are compared with the total amounts accumulated in the sap, computed on the basis of the estimated total volumes of sap, it is evident that normally more than 80 per cent., and sometimes more than 90 per cent., of the total amounts of these elements absorbed by the roots are present in the sap. An increase in the total salt concentration of the sap is accompanied by a parallel increase in electrical conductivity. The values cited may, of course, be too low if the unrecovered sap has a higher concentration than that of the expressed sap. The amount of nitrate absorbed from solution was nearly always much in excess of the amount present in the sap, because of the reduction of nitrate in the tissues. When bases enter the cell in association with nitrate and the latter is subsequently reduced, direct evidence shows that the basic residue is neutralized by organic acids.

Experimentation

REPRODUCTION OF RESULTS

By proper control of all factors, it is feasible to obtain a very close agreement of results on replicate sets of roots, each of which represents a large number (168) of selected seedlings. Evidence of this is found in table I, in which results are reported for roots examined after a period of absorption from a KBr solution. The plants for the experiment supplying the data for table I were grown from December 1 to December 22 in the standard nutrient solution. Each set of root systems, consisting of 168 plants each, was then placed in a shallow, covered tray filled with 3800 cc. of an external solution having an initial composition of 0.0025 M $Ca(NO_3)_2$, 0.005 M KNO_3, 0.001 M $MgSO_4$, and 0.0005 M KH_2PO_4, and remained in the solution for an absorption period of 27 hours. In this

TABLE I

EXAMPLE OF REPLICATION OF RESULTS IN SALT ACCUMULATION EXPERIMENTS WITH EXCISED ROOTS*

CONDITION	SET	CONCENTRATION OF K IN EXPRESSED SAP IN MILLIEQUIVALENTS PER LITER	K ACCUMULATED IN SAP IN MILLIEQUIVALENTS PER LITER
Initial	A	23.7	
	B	22.9	
At end of absorption period	A	85.0	
		85.5	62.0
	B	83.5	
		85.0	61.0
	C	87.5	
		88.0	64.4
	D	85.0	
		85.0	61.7
			Average..... 62.3

* Results of duplicate analyses on samples of sap are also shown.

experiment, the period of absorption was sufficiently long to permit the cells to reach a concentration of salt approaching the maximum attainable under the favorable conditions provided. In some experiments, agreement, although entirely adequate for present purposes, may not be quite so good as that just indicated, when conditions are unfavorable for salt accumulation, or when intermediate periods are involved. Most of the experiments were carried out in duplicate, (several examples are given in tables) and important deductions were checked by repetition of entire experiments at different times and in different ways. Positive conclusions are derived only from consistent effects of such large magnitude that their statistical significance is not open to doubt. Quantitative comparisons are always made within a single experiment, between sets of roots derived from the same uniform population of seedlings.

ANALYTICAL TECHNIQUE

At various times and in accordance with the purpose of the experiment, the following analytical methods have been utilized in the general investigation:

1. Bromide according to general procedure of HIBBARD (9). Ignition was carried out with NaOH at 600° C. Thiosulphate was checked against standardized solutions of dichromate, or in recent experiments by a modi-

fied technique in which the reagents were standardized with a KBr solution, and all titrations limited to volumes of 2 to 5 cc., maintaining a slow and uniform rate of aeration.

2. Total halide by Mohr's method.

3. Potassium by volumetric cobalti-nitrite procedure of HIBBARD and STOUT (**10**).

4. Calcium and magnesium by the McCrudden method.

5. Sodium by uranium acetate method (**1**).

6. Conductivity by Kohlrausch bridge method with ear-phones and amplifier system; freezing-point depression by use of a Beckmann thermometer.

7. Total and nitrate nitrogen by modified methods of PUCHER *et al.* (**30**) and VICKERY and PUCHER (**41**).

8. Sugar (total) by Munson-Walker method.

9. Organic acids by electrometric titration of sap or by separation of non-volatile ether-soluble acids followed by titration (method to be published elsewhere by A. ULRICH).

10. Carbon dioxide evolved by absorption in NaOH and double titration, using Winkler method with use of $BaCl_2$.

11. pH and buffer curves of sap, using the hydrogen electrode. Recent experiments have shown the value of the glass electrode for future work. pH values of external solutions estimated by standard indicator methods.

12. Bicarbonate by direct titration, also by driving off CO_2 from acidified solution into NaOH solution.

13. Inorganic SO_4 and PO_4 by direct precipitation and with standard methods of analysis.

The errors of duplicate analyses on sap or culture solutions never approached the orders of magnitude to which significance is attached.

HYDROGEN ION EFFECTS

The interrelations between hydrogen ion concentration of the external solution and salt accumulation require a separate later discussion. The general aspects of salt accumulation can be considered now without particular reference to the hydrogen ion factor. We need only say, at this point, that our data emphasize that the effects of the metabolic activities of root cells on the hydrogen ion concentration of the external solution may be of greater moment than the effects of hydrogen ion concentration of the solution, *per se*, on the accumulation of salt, within a wide range of pH.

EFFECTS OF AERATING ROOT TISSUES ON ACCUMULATION OF SALT

It has been proved by STEWARD (**35**) that accumulation of potassium salts by discs of storage tissues occurs only when oxygen is supplied to the

tissues at a suitable tension and rate of flow of the gas stream. Active aerobic respiration was found to be indispensable. ROSENFELS (31) has shown that rapid accumulation of Br by *Elodea*, in the dark, is likewise dependent upon aeration. In the light, the necessary oxygen is provided by photosynthetic processes and this is also true of *Nitella*.

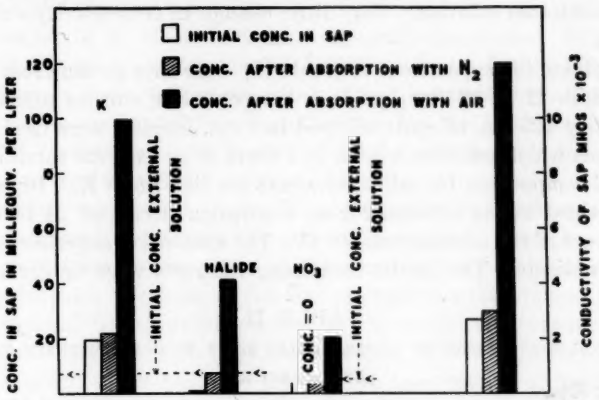

FIG. 3. Effect of aeration on accumulation of salt by excised roots of barley plants.

A general demonstration of the effects of aeration on salt accumulation by root tissues was made in a very direct and simple manner (fig. 3).

A description of the experiment supplying the data for figure 3 follows. The plants (168) of each set were grown in a complete culture solution from May 31 to June 26. Each set of the excised roots was then placed in 3 liters of an external experimental solution consisting of 0.0075 M KBr, and 0.0025 M $Ca(NO_3)_2$, and remained in this solution for an absorption period of 10 hours. The temperature of the solution was kept at 24° C.[2] During the absorption period, a rapid stream of air was passed through one solution and purified nitrogen gas through the other. The nitrogen gas, however, retained traces of oxygen. Some oxygen was also present in the tissues and solution at the beginning of the absorption period. The pH of the initial culture solution was 6; of the final, with air passing through it, 5.8, and with N_2 passing through it, 6.3.

Note should be made of the fact that much of the NO_3 absorbed was reduced, and that K absorbed in association with NO_3 remained in the sap in equilibrium with organic acid anions formed in the course of metabolism.

When a stream of air was passed through the external salt solution, K, Br, and NO_3 accumulated rapidly in the sap, against steep concentration

[2] Except where otherwise noted, temperatures were maintained in bath within ± 0.2° C.

gradients. Conductivity of the expressed sap increased about 500 per cent. On the other hand, when nitrogen gas (containing as an impurity approximately 0.2 per cent. oxygen) was substituted for air, no NO_3 was found in the sap, and K was increased in concentration but very slightly. Br gained entrance to the sap, but only to an approximate equality of concentration with the external solution. Very little change in conductivity occurred in this case.

The plants for experiment A (table II) were first grown from January 11 to March 21 in 20-liter jars in a low-potassium culture solution. Approximately 125 gm. of roots, clipped in 5 cm. lengths, were used for each set of roots which was then placed in 3 liters of an external solution having an initial composition (in milliequivalents per liter) of 5 K, 6 Br, and 1 Ca, and remained in the solution for an absorption period of 24 hours. The temperature of the solution was 20° C. The absorption experiment was run in quadruplicate. The bottles containing the roots were tightly stoppered

TABLE II

ACCUMULATION OF SALT BY EXCISED BARLEY ROOTS WITH AND WITHOUT OXYGEN

EXPERIMENT A

CONDITION	COMPOSITION OF SAP		ACCUMULATED IN SAP	
	K	HALIDE	K	HALIDE
	MILLIEQUIVALENTS PER LITER*		MILLIEQUIVALENTS PER LITER*	
Initial	15.4	0.7		
After absorption period with stream of air	72.5	57.2	57.1	56.5
After absorption period without aeration	17.2	10.2	1.8	9.5

EXPERIMENT B

CONDITION	SET	COMPOSITION OF SAP			ACCUMULATED IN SAP		
		K	Br + Cl	NO_3	K	Br + Cl	NO_3†
		MILLIEQUIVALENTS PER LITER			MILLIEQUIVALENTS PER LITER		
Initial		28.7	1.6	0.1			
After absorption period with N_2	A	27.9	7.0	0.1	−0.8	5.4	0.0
	B	27.7	6.4	0.0	−1.0	4.8	−0.1
After absorption period with air	A	89.3	30.4	21.6	60.6	28.8	21.5
	B	81.3	30.8	20.5	62.6	29.2	20.4

* All values were within 10% of the averages given.

† Some NO_3 was absorbed and reduced. Values given are for NO_3 ion.

during the absorption period, but the solution and tissues were not initially free of oxygen. Four other sets were treated in the same way except that a stream of air was passed through the solutions. Again the effect of aeration is evident. The oxygen initially present in the solution or in the tissues maintained the cells in apparently healthy condition for the period in question and permitted a small net accumulation of Br and a very slight net accumulation of K, which, however, may not be significant. In marked contrast, the aerated tissues accumulated large amounts of K and Br. The concentrations of these ions in the sap attained values four or five times higher than the corresponding concentrations for the unaerated tissues. Prolonging the experiment would doubtless have resulted in a loss of salt from the unaerated tissues.

Still another experiment (table II, experiment B), conducted under different conditions, has a similarly clear significance. Each set of 168 plants for experiment B was grown in the standard nutrient solution from April 22 to May 13. Each set of the root systems was then placed in 3800 cc. of an external experimental solution having an initial composition (in milliequivalents per liter) of 10 K, 5 Br, 9 NO_3, and 4 Ca, and remained there for an absorption period of 9 hours. The temperature of the solution was 23.6° C. A gas stream was passed through the solution. The pH of the external solution varied from the initial of 5.6 to a final of 6.5 for air treatment; and from 5.6 to 7.8 for N_2 treatment. The roots in the solution through which nitrogen gas was passed did not accumulate any K or NO_3 (although considerable NO_3 was absorbed from solution and rapidly re-

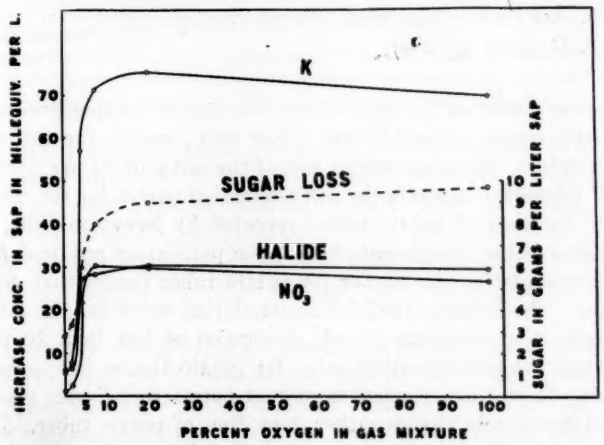

FIG. 4. Relation of oxygen tension in flowing gas stream to accumulation of salt by excised barley root systems.

duced) and halide concentration in the sap rose to a value only slightly in excess of that of the external solution, while the aerated tissues, in the 9-hour period, accumulated large amounts of all three ions, K, Br, and NO_3. The internal concentrations of the latter ions far exceeded the external at the end of the period of salt absorption.

One experiment had for its purpose a study of the effects on salt absorption of varying oxygen tensions, in a rapidly flowing gas stream (fig. 4). The plants, consisting of 168 for each set, were first grown in a complete culture solution from March 29 to April 24. Each set of root systems was then placed in 4-liter bottles containing 3 liters of an external solution having an initial composition of 0.005 M KBr, 0.005 M KNO_3, and 0.002 M $Ca(NO_3)_2$, and remained there for an absorption period of 10½ hours.

Mixtures of oxygen and nitrogen gases were made in cylinders under compression, and the composition of the mixtures determined by analysis. The gas mixtures used were as follows:

Gas	Oxygen content
	%
1. N_2 purified with pyrogallol	0.2
2. N_2 unpurified, from cylinder	1.7
3. $N_2 + O_2$ mixture	4.1
4. $N_2 + O_2$ mixture	7.5
5. Air from compressor, filtered through water	
6. O_2 from cylinder	

The values recorded in figure 4 are averages of duplicate experiments in which the results agreed within 5 per cent., except for the very low absorption values, where agreement was of the order of 10 per cent.

It is of interest to compare the salt absorption curves for the root tissues with those for discs of potato tuber, reported by STEWARD (35). Under the conditions of the experiments the oxygen percentage required for maximum Br accumulation was higher for potato tuber tissues than for barley root tissues. The bromide (halide) accumulation curve for the latter rises very sharply to a maximum of salt absorption at less than 10 per cent. oxygen, while the corresponding value for potato tissues is approximately 20 per cent. Recent experiments show that potato root tissues resemble in this regard barley root tissues rather than discs of potato tuber. Data are also presented for K and NO_3 accumulation, and sugar loss, by the barley root tissues. All the curves follow a very similar trend. The experiment

was concerned with only one type of root system and it will become of interest to conduct similar experiments on root systems of different initial status with regard to salt and sugar content. Research should also be done with reference to possible effects of manganese, copper, zinc or other elements required in minute quantity.

Respiration and salt accumulation

Respiration data were not obtained in the root experiment on the effects of oxygen tensions, but the experiment of Steward (35) on potato tuber tissues provides evidence that the curves for CO_2 production and for oxygen tension assume the same form. Respiration data from other experiments on root tissues are presented in tables III and IV.

TABLE III

Carbon dioxide production by tissue as influenced by oxygen supply*

Condition	Mg. CO_2 per gm. fresh tissue per hr.	Relative to air as 100
	mg.	
Excised barley roots with air	0.734	100
" " " " N_2†	0.282	38
Discs of potato tuber‡ with air	0.183	100
" " " with N_2 containing 2.9 per cent. O_2	0.086	47

* Compare also table 4.

† The nitrogen gas was compressed in a cylinder and usually contains about 2 per cent. of O_2.

‡ The potato tuber results are from Steward (35), table 5, page 217.

The barley plants used, in the experiment determining CO_2 production by tissues as influenced by oxygen supply (table III), were grown in a standard nutrient solution from April 22 to May 12. Each root system was placed in 3 liters of an external solution, having an initial composition of 0.005 M KBr, 0.005 M KNO_3, and 0.002 M $Ca(NO_3)_2$, and remained there for an absorption period of 9 hours. The temperature of the solution was 23.5° C.

The relative values for CO_2 production are in general agreement with the results given by Steward (35) and Steward and Broyer (39) on potato tuber tissues. The same broad conclusion seems to apply to every kind of tissue so far studied with reference to the relation between respiration and salt accumulation, namely, that CO_2 production (aerobic) by tissues capable of marked salt accumulation, reflects metabolic activities essential to

TABLE IV

REVERSIBILITY OF EFFECT ON SALT ACCUMULATION OF PASSING NITROGEN THROUGH SOLUTION

CONDITION	COMPOSITION OF SAP			INCREASE OF CONCENTRATION IN SAP			RESPIRATION RATE*
	K	Br	CONDUCTIVITY	K	Br	CONDUCTIVITY	Mg. CO_2 / Gm. tissue × hr.
	MILLIEQUIVALENTS PER LITER		MHOS. $\times 10^{-3}$	MILLIEQUIVALENTS PER LITER		MHOS. $\times 10^{-3}$	
Initial	29.7	0.0	3.97				
After absorption with air 6 hrs.	61.3	26.0	7.31	31.6	26.0	3.34	0.422
After absorption with N_2 6 hrs.	28.9	3.3	3.95	− 0.8	3.3	−0.02	0.213
After absorption with air 6 hrs. ... following 6 hrs. with N_2	53.2	17.3	6.47	23.5	17.3	2.50	0.320

* The respiration rate is the average for the period involved.

this process, but without a stoichiometric relation between amount of salt accumulated and amount of CO_2 produced.

Is the effect of an inadequate supply of oxygen in preventing salt accumulation irreversible? This question we have sought to answer by subjecting root tissues to a stream of purified nitrogen gas for six hours and then changing the gas from nitrogen to air. The plants used in this experiment (table IV) were grown in the standard nutrient solution from October 30 to November 21. The root tissues were then placed in 3 liters of an external solution having an initial composition (in milliequivalents per liter) of 7.5 KBr, and remained there during the absorption period. A stream of gas was passed through the solution. The nitrogen gas was passed through sodium pyrogallate to remove the oxygen. CO_2 was removed from the nitrogen and from the air by passing the gas through sodium hydroxide. The temperature of the solution during the absorption period was $20° \pm 0.5°$ C.

Within the period designated, nitrogen gas completely suppressed salt accumulation (Br was absorbed but the sap concentration was lower than that of the external solution), yet this process was resumed when oxygen was later supplied to the same tissues, although at a somewhat diminished rate. It is significant that the rate of respiration was likewise diminished (table IV).

Incidentally, this experiment also illustrates that the *mere production of* CO_2 by roots does not explain salt accumulation as certain theories would suggest. There are involved rather metabolic activities associated with aerobic respiration (**36**).

All our experiments lead to the conclusion that oxygen must be supplied to barley roots to maintain the cell activities associated with accumulation of salt, or the retention of the salt already accumulated, over an extended period. It is not yet so certain to what extent it is essential to lower the carbon dioxide content of the medium. Several preliminary experiments on root tissues, using a complete nutrient solution, showed no important retardation of salt accumulation when an air-CO_2 mixture containing 10 per cent. of CO_2 by volume was passed through the solution. An admixture of even 20 per cent. carbon dioxide did not produce a very great effect under the conditions of the experiment. This general question, however, demands further research.

The absence of aerobic metabolism associated with rapid salt accumulation does not necessarily mean that the cell becomes impermeable to a salt. Bromide may enter the sap to approximate equality of concentration with the external solution under anaerobic conditions. The reason nitrate apparently does not always behave in the same way is that the nitrate can be reduced as it is absorbed. Attention is called to the fact that a completely anaerobic condition was not attained in any of the experiments. There was a slight oxygen impurity in even the purified nitrogen gas and some oxygen must have been initially present in the tissues or solutions. This

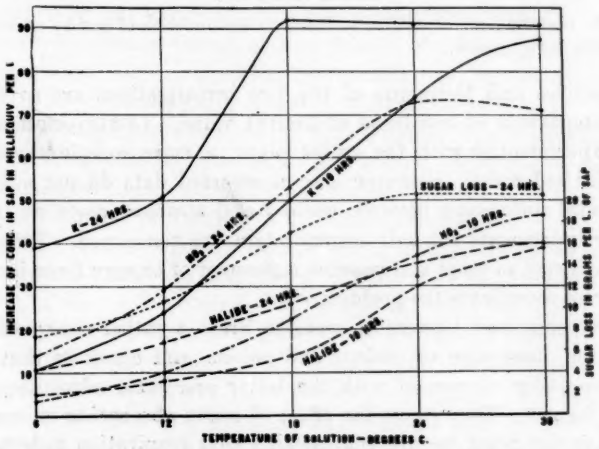

FIG. 5. Relation of temperature and time to salt accumulation by excised young barley root systems.

may explain slight accumulations of salt, or failure of tissues to lose potassium, during a brief period, when nitrogen gas is passed through the culture solution.

The experiments recently reported by PETRIE (**27**) on entire plants have led him to conclude from statistical evaluation of results that aeration of the root medium accelerates absorption of salts although, as PETRIE recognizes, in experiments of this type, conducted over an extended period, it is difficult to relate the effects of oxygen directly to the process of salt absorption. Data are not reported on the extent of salt accumulation in the root sap.

The studies of LUNDEGÅRDH (**18**), and LUNDEGÅRDH and BURSTRÖM (**19–22**) on respiration in relation to salt absorption were undertaken from a point of view different from that of STEWARD or of the present writers.

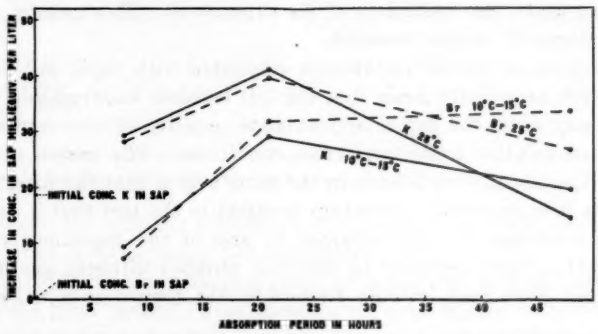

FIG. 6. Relation of temperature and time to accumulation of salt by excised root systems of older barley plants.

The objectives and technique of the two investigations are so dissimilar that a comparison of results is of limited value. LUNDEGÅRDH and BURSTRÖM experimented with the entire plant, a more complex system than that of excised roots. However, our unreported data do not suggest any fundamental differences between excised and attached roots, as far as the general requirements for salt accumulation are concerned. Translocation of salt from root to shoot and possible movement of oxygen from illuminated shoot to root complicate the problem.

LUNDEGÅRDH and BURSTRÖM seem to draw a rather sharp distinction between the absorption of cations and anions, and conclude that respiration is especially concerned with the latter and some adsorption process with the former. They stress the effect of anion absorption on respiration rather than the point we are emphasizing that respiration reflects energy exchanges essential to accumulation by root cells of *both* cations and anions.

The experiments reported by LUNDEGÅRDH and BURSTRÖM were confined to the removal of salt from the culture solution, so that it is uncertain if they were dealing with salt accumulations in the sap of magnitudes comparable to those of the present experiments. It is conceivable that the environmental conditions, in their experiments, especially that of light, did not permit the production of root systems of high capacity for salt accumulation. The question also arises whether the aeration was fully adequate for the most rapid rate of salt absorption, or the attainment of high levels of salt concentration in the root sap. The initial salt status of the roots was not stated and, as has been indicated, this is an important factor determining salt absorption during a limited experimental period.

With regard to the relation of anaerobic conditions to anion absorption and to respiration, we have noted that nitrate may be removed from solution to an appreciable extent under conditions which are not sufficiently aerobic to permit accumulation of nitrate as such in the sap. In this connection it may become necessary to give consideration to bacterial activities when the environment is unfavorable to aerobic root metabolism, and absorption is studied over prolonged periods. We desire to postpone further discussion of the effects of salts on respiration, awaiting additional evidence.

TEMPERATURE EFFECTS

The principle that salt accumulation is dependent upon metabolic activities of cells, suggests that the process of accumulation should have a high temperature coefficient over certain ranges of temperature. This expectation is fulfilled by the experimental evidence on roots, which is in accord with evidence on storage tissues and on *Nitella* and *Elodea* cells (**16, 31**). The Q_{10} values for salt accumulation by excised roots are of a high order (2.5 to 5.0) over a considerable range of temperature (6 to 24° C.), and similarly high temperature coefficients were obtained in comparable studies with entire barley plants. The data from several experiments on excised roots are presented in figures 5 and 6, and tables V and VI.

In the experiment performed to determine the relation of temperature and time to salt accumulation by excised young barley root systems (fig. 5), each set of plants (168) was first grown in a complete culture solution from February 8 to March 2. Each set of the excised roots was then placed in shallow covered trays containing 3800 cc. of an external culture solution having an initial composition of 0.005 M KBr, 0.005 M KNO_3, and 0.002 M $Ca(NO_3)_2$, and remained there for an absorption period of 10 or 24 hours.

The data pertaining to NO_3, given in the graph (fig. 5), refers to NO_3 accumulation as such in the sap. Additional nitrate was also absorbed and reduced in the tissues.

The plants in the experiment performed to determine the relation of temperature and time to accumulation of salt by excised root systems of older barley plants (fig. 6), were first grown in large tanks containing a low-potassium culture solution from December 21 to March 4. Each sample of root tissues then taken was composed of 75 gm. (fresh weight) of tissues obtained from a large mass, prepared by clipping many uniform root systems into approximately 5-cm. segments. This root material was placed in bottles containing 3800 cc. of external culture solution having an initial

TABLE V

TEMPERATURE EFFECTS ON SALT ABSORPTION BY EXCISED ROOTS OVER PROLONGED PERIODS

TEMPERATURE	PERIOD	REMOVED FROM SOLUTION	
		K	Br
		MILLIEQUIVALENTS PER LITER	
°C.	hours		
10	73	4.56	3.94
10	124	3.81	4.19
21	73	1.32	3.04
21	124	−0.36	0.53

TABLE VI

ACCUMULATION RATIOS FOR POTASSIUM, NITRATE, AND HALIDE IN EXCISED BARLEY ROOT SYSTEMS

TEMPERATURE	COMPOSITION OF CULTURE SOLUTION			COMPOSITION OF SAP			ACCUMULATION RATIOS*		
	K	NO_3	HALIDE	K	NO_3	HALIDE	K	NO_3	HALIDE
	MILLIEQUIVALENTS PER LITER			MILLIEQUIVALENTS PER LITER					
°C.									
6	9.62	8.35	4.84	34.7	4.8	5.9	3.6	0.6	1.2
12	9.32	8.27	4.76	47.7	11.1	8.8	5.1	1.3	1.9
18	8.45	7.75	4.64	73.2	23.7	14.6	8.7	3.1	3.1
24	7.98	7.29	4.30	97.8	38.1	30.9	12.3	5.2	7.2
30	7.42	6.70	3.96	112.0	47.8	39.9	15.1	7.1	10.1

* Accumulation ratio is the concentration in sap divided by concentration in external solution (final).

composition (in milliequivalents per liter) of 5 K, 6 Br, and 1 Ca, and remained there during the absorption period.

The results of the temperature effects on salt absorption by excised roots over prolonged periods are given in table V. The plants for this experiment were grown in 20-liter jars, containing 25 plants each, from January 28 to March 15. The culture solution in which these plants were grown consisted of 0.005 M $Ca(NO_3)_2$, 0.005 M KNO_3, 0.002 M $MgSO_4$, and 0.001 M KH_2PO_4. Excised entire root systems of 25 plants were used for each absorption treatment. Each set of these roots was next placed in 3 liters of an external solution having an initial composition (in milliequivalents per liter) of 5 K, 6 Br, and 1 Ca, and remained there during the absorption period.

The set up of the experiment which provided the data for table VI was the same as that described for figure 5. The absorption period was 10 hours.

It is important to emphasize that the temperature effects apply to both cations and anions. STEWARD has pointed out that certain earlier conclusions from experiments on storage tissues (**26**)—that temperature acceleration of salt absorption may be restricted to anions—is possibly explained on the ground that the tissues were not in a highly active state of metabolism, because of insufficient aeration.

While high temperature coefficients are, in fact, characteristic of salt accumulation by roots like those used in our experiments, to establish this conclusion demands the correct experimental conditions. In preliminary work we were unable to obtain satisfactory evidence of temperature effects, for reasons which subsequently became clear. It is essential to do the experiments with tissues in which active cells of high potentiality for salt accumulation predominate. As has already been suggested, the losses of salts by inactive or senescent cells in older root systems may partially or wholly obscure the accumulation by active cells, since the whole mass of tissues, or the composite culture solution, is under examination. The cells should have an initially low salt content, and especially in the absence of rate studies, the effect of temperature upon their salt absorption should be determined only during periods when the cells remain capable of continued increase in salt concentration. Furthermore, in a long period available carbohydrates may be depleted or other changes occur in the tissues unfavorable to salt accumulation or retention. These changes are accelerated with increasing temperatures and when a sufficient interval of time has elapsed, the accumulation of salt may be depressed by the secondary effects of temperature, or some cells may begin to lose salt (table V and fig. 6). In long experiments the apparent paradox may be presented that a greater salt content is obtained at the lower temperature. Unless properly interpreted such results confuse the effect of temperature upon salt accumula-

tion with other effects of temperature. The young low-salt, high-sugar tissues, developed according to the technique already described, are well suited to the study of temperature acceleration of salt accumulation, but time effects and sugar depletion must be taken into consideration.

The temperature effects in question concern primarily K, Br, and NO_3 ions. Temperature data have been obtained on the removal of Ca, Mg, SO_4, and HPO_4, or H_2PO_4 from culture solutions and these suggest that high temperature coefficients for absorption apply also to these ions. However, special difficulties are found in the interpretation of the results. The amounts of Ca, Mg, or SO_4 absorbed in a brief period are usually very small, and questions of precipitation of calcium and magnesium salts on root surfaces arise, especially when secondary hydrogen ion changes occur in the solution.

Whether temperature coefficients are the same for the accumulation of all ions cannot be decided without greater knowledge of the mechanism of salt accumulation. Adequate rate studies remain to be carried out. Also ionic exchanges versus simultaneous uptake of cation and anion and, in a mixed salt solution, complex interionic effects are involved. Reduction of NO_3 in the tissues is another complicating factor. Temperature effects on salt accumulation are, of course, indirect in the sense that it is the metabolism of the plant which is being affected, one of the results of accelerated metabolism being accelerated salt absorption, provided that other conditions favor this process.[3]

ACCUMULATION RATIOS.—One of the temperature experiments also affords information concerning the magnitude of accumulation ratios for potassium, nitrate, and halide (ratio obtained by dividing concentration in expressed sap by concentration in external solution). These ratios rise to approximately 15 for potassium, 7 for nitrate, and 10 for halide (table VI). The values apply to culture solutions containing over 7 milliequivalents per liter of potassium salt. Far higher accumulation ratios are easily obtainable by utilizing more dilute solutions. They may reach values in excess of 1000. In fact, in some tests the root tissues accumulated potassium to a high concentration, and left only a trace in the culture medium.

SALT STATUS OF TISSUES

It has been pointed out that the capacity of root tissues to accumulate salt over a given period is dependent upon the salt supply previously

[3] Further study should be made of the translocation of solutes from root to shoot. In our experiments with excised roots the conditions were so chosen that temperature effects on salt accumulation could be easily demonstrated. We feel that these effects are inherently characteristic of the process of accumulation, but appreciate how many complicating factors enter into the study of salt absorption by entire plants grown under diverse conditions.

accumulated by the tissues. By frequent changes of external culture solution in the preliminary growth period, the supply of nutrients is renewed and this leads to the production of root tissues with a very high salt content, since uptake of salt is in excess of translocation to the shoot. The excised high-salt root tissues had a very limited capacity for absorbing additional salt and in some cases no K was removed from solution or even a slight loss from the tissues as a whole occurred, although a small amount of anion, NO_3 or Br, could be absorbed by a process in effect equivalent to an exchange of these anions for bicarbonate or organic acid anions derived from the tissues (table VII, experiment A). The actual mechanism of salt accumulation of this type has not yet been elucidated.

TABLE VII

RELATION OF INITIAL SALT STATUS OF ROOT SYSTEM TO ABSORPTION OF SALT IN SUBSEQUENT PERIOD

EXPERIMENT A

CONDITIONS OF EXPERIMENT	ABSORBED FROM SOLUTION	
	K	Br
	MILLIEQUIVALENTS PER LITER	
High-salt, excised roots	−0.20	0.50
Low-salt, " "	2.66	1.90

EXPERIMENT B

CONDITIONS OF EXPERIMENT	ABSORBED FROM SOLUTION	
	K	NO_3
	MILLIEQUIVALENTS PER LITER	
Entire plants	2.64	2.56
Excised root systems	2.53	2.43

EXPERIMENT C

CONDITIONS OF EXPERIMENT	ABSORBED FROM SOLUTION	
	K	NO_3
	MILLIEQUIVALENTS PER LITER	
High-salt, entire plants	2.05	2.40
" " , excised root systems	0.67	0.89
Low-salt, entire plants	3.66	3.49
" " , excised root systems	3.02	2.93

The sets of plants, consisting of 168 plants each, for experiment A (table VII) were grown in the standard culture solution from May 23 to June 15. For the low-salt sets, the culture solution was not changed during the growth period; for the high-salt sets, the culture solution was renewed daily from May 29 to June 14 inclusive. The roots of each set were then placed in 3 liters of an external solution having an initial composition (in milliequivalents per liter) of 7.5 K, and 7.5 Br, and remained there for an absorption period of 20 hours. The solution also contained small amounts of carbonates and chlorides of Ca and Mg. The pH of the culture solution was modified during the experimental period from 7.5 initial to 8.1 for the high-salt, and from 7.5 to 7.3 for the low-salt condition. The temperature of the solution was 25° C.

The very different behavior of low- and high-salt tissues is illustrated in table VII. During the given periods of salt accumulation, far more salt was absorbed by the low-salt roots than by the high-salt roots and the relation between cation and anion absorption was also altered to some extent.

It is not the intention of the writers to discuss at this time the problem of translocation of salt in relation to accumulation, but a comparison of the absorption of salt by excised root systems and by entire plants, for low- and high-salt plants, is of immediate interest. (table VII, experiments B and C).

The sets of plants, consisting of 168 plants each, for experiment B (table VII) were grown in shallow trays containing the standard nutrient solution from April 28 to May 17. Each set of roots was then placed in 3800 cc. of an external solution having an initial composition of 0.0025 M $Ca(NO_3)_2$, 0.005 M KNO_3, 0.001 M $MgSO_4$, and 0.0005 M KH_2PO_4, and remained there during an absorption period of 7 hours. The temperature of the solution was was 23.5° C.

The sets of plants, consisting of 168 plants each, of experiment C (table VII) were first grown in standard nutrient solution from June 10 to June 29 as in experiment A. The culture solution of the low-salt sets was not changed during the growing period; that of the high-salt sets was renewed daily from June 18 to June 28 inclusive. Each set of the excised roots was then placed in 3800 cc. of an external solution having an initial composition of 0.0025 M $Ca(NO_3)_2$, 0.005 M KNO_3, 0.001 M $MgSO_4$, and 0.0005 M KH_2PO_4, and remained there during the absorption period of 7 hours.

Over the limited period of the experiments, the amount of salt absorbed by the roots excised from the low-salt plants was almost as large as the amount absorbed by the entire plants, while the entire high-salt plants had a much higher absorbing capacity than did the corresponding excised root systems. The removal of salt from root to shoot permitted more salt to enter the

root system. Despite this influence of the shoot, more salt was absorbed by the excised root systems of the low-salt plants than by the entire high-salt plants, even though the latter were considerably larger than the low-salt plants. One cannot doubt the importance of knowing the salt status at a given time, of either excised or attached roots. Incidentally, these experiments, supported by many others, illustrate that the process of accumulation of salt by root cells has no direct dependence on transpiration, but the continuance of salt absorption is influenced by transpiration to the extent that the latter may be a factor in determining the upward movement of salt, a point to be discussed in another article.

A clearer understanding can now be gained of the effects of illumination on the absorption of salt. If, during the day period, the translocation of salt out of the root system is rapid enough partially to deplete the root, then, in a subsequent dark period, large absorption of salt can occur under conditions favorable for the metabolic activities of root cells.

Relations between cation and anion accumulation

Time effects are an important consideration in the study of salt accumulation by excised roots (fig. 7). In the experiment, for making this study, the plants were first grown in large jars containing a large reserve of culture solution per plant from November 5 to January 6. The method used, of cutting root systems into segments, was the same as that described for the experiment for figure 6. The absorption experiment was carried out in bottles containing 3800 cc. of culture solution, and 75 gm. of root tissue. The initial composition of the external solution for the absorption period consisted of 0.002 M KBr, and 0.0002 M $CaBr_2$. The temperature of the solution was 20° C.

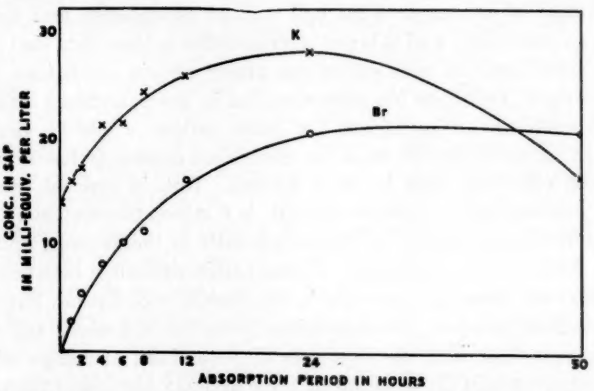

Fig. 7. Time effects on accumulation of salt by excised barley root systems approximately 9 weeks old.

In a number of the experiments on root tissues possessing a high potentiality for salt absorption, K and Br were accumulated in the sap, or withdrawn from solution, in nearly equivalent quantities (figs. 6, 7, early period, and unpublished results). This type of absorption has been designated by STEWARD as "primary" absorption and in some respects forms the basic problem of accumulation. However, various secondary effects complicate the study of this relation and it is apparent that actual equivalent absorption by root tissues of cation and anion of the salt under study can be demonstrated only under special conditions, some of which are still obscure. Nearly equivalent absorption of K and Br may be found over one period of time, while, over a longer period, the same tissues may give results which seem to indicate a larger absorption of Br than K (figs. 6, 7). The explanation probably is that when the activity of the tissues declines, some cells lose K, accompanied by bicarbonate or organic acid anions in excess of the loss of Br. The excess accumulation of K over Br found in some experiments to be reported later requires a further study of the possibility that HCO_3 ions may be involved in this process.

Discs of potato tissues during an initial period lose K, Ca, and Mg, together with organic acid anions, followed by a period of reabsorption. In this later period, K and Br may be absorbed in approximately equivalent quantities (**32**). Young root tissues show no appreciable initial loss of potassium ions but small amounts of calcium and magnesium ions enter the culture solution, accompanied by bicarbonate, phosphate, or organic acid anions. Some evidence suggests that the calcium and magnesium salts may be derived in part from surface deposits on roots. It is possible that potassium may be subsequently absorbed by active cells together with these released anions. Also, earlier work by various investigators supports the view that exchange of cations between cell wall or protoplasm and the culture solution can take place, and it is not unreasonable to postulate that hydrogen ions may participate in such exchanges under certain conditions, resulting in an increase of hydrogen ion concentration in the unbuffered culture solution. An exchange of potassium for other cations would be particularly expected in an initial period, when the tissues are extremely low in potassium content and relatively high in other cations. But, in general this type of exchange process, with respect to cations, is a minor phenomenon compared with the active accumulation of potassium salts in the vacuole, and the two processes should not be confused. Direct cation exchange between solution and vacuolar sap remains uncertain in the case of root tissues, but since the losses of sodium, calcium, or magnesium from the expressed sap are very small in comparison with the increases of potassium, exchange with these cations cannot explain the behavior of these actively absorbing tissues.

From solutions of $CaBr_2$ or $Ca(NO_3)_2$, or mixed solutions of potassium and calcium salts, anions can sometimes be accumulated by root systems in

much greater equivalent quantity than cations, and NO_3 may be absorbed by roots initially high in K in greater equivalent quantity than K from KNO_3 solutions. As already suggested, the effect is that of an exchange of HCO_3 ions for Br or NO_3 ions. This process is also dependent upon aerobic metabolism of the cell according to our experimental data. Under anaerobic conditions little or no accumulation of anions in the sap occurred even though considerable amounts of CO_2 were evolved (fig. 3 and table II). The absorption of NO_3 from solution with concurrent reduction presents another phase of the general problem. A detailed study of cation-anion accumulation with a series of salts is now being undertaken.

Available carbohydrate and accumulation of salt

Inasmuch as a high rate of respiration is associated with rapid accumulation of salt by root tissues, the available carbohydrate supply is of importance. A rapid loss of sugar occurs when root tissues are aerated as in the absorption procedure, although generally more sugar is lost than is accounted for by carbon dioxide liberated, and no simple relation is found between sugar concentration and CO_2 evolved. A number of experiments have been carried out in which the excised roots were largely depleted of sugar by vigorous aeration, while immersed in tap water or a dilute calcium sulphate solution, preceding the period during which the absorption of potassium salts was studied. It is desired to extend these experiments before drawing final conclusions, but the results now available indicate that root tissues sufficiently reduced in sugar content suffer a large decrease in their capacity to accumulate salts, which can be restored, at least in part, by supplying sugar to the roots through the culture solution. Apparently it is necessary to reduce the sugar concentration to a low value before salt absorption declines significantly. In an article following this one, Prevot and Steward (**28**) have presented data on segments of primary roots of barley which also indicate that accumulation of Br by root cells low in sugar is accelerated by providing a supply of sugar in the external solution during the period of salt absorption.

The high-salt roots have a markedly lower initial sugar content than the low-salt roots, and this is significant in certain experiments, especially those carried on for a number of days. However, various lines of evidence, including the time effects described in the next section, suggest that the limitation of salt absorption by high-salt roots, in most of the experiments now under consideration, cannot be ascribed merely to deficiency of carbohydrate. The root cells must have a certain upper limit for salt concentration even when they remain in an active state of metabolism. Conceivably maximum distention of the cell wall or a salt saturation of protoplasmic constituents limits salt absorption, but the mechanism is still obscure.

Time effects

Preliminary experiments using an earlier technique, with older root systems of moderate potentiality for salt accumulation, had shown that absorption of salt (KBr) by excised roots reached an approximate maximum within 24 hours, after which a net loss of potassium from the tissues as a whole took place, without concurrent loss of Br, within total period of 48 hours (fig. 7). More recently a time experiment was carried out with young root systems, according to the principal technique outlined earlier in this article (figs. 8, 9).

The plants, consisting of 168 in each set, were first grown in a complete culture solution from June 30 to July 19. Each set of roots was then placed in a 4-liter bottle containing 3 liters of an external solution having an initial

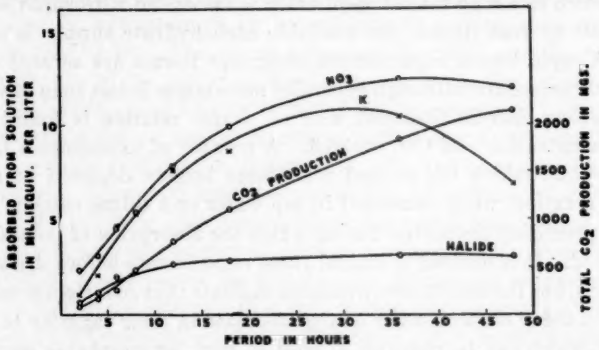

Fig. 8. Time effects on absorption of salt by young excised barley root systems, based on analysis of external solutions.

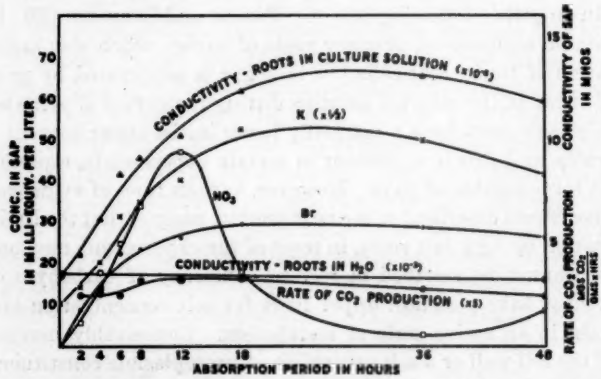
CONC. IN SAP IN MILLI-EQUIV. PER LITER
CONDUCTIVITY OF SAP IN MHOS
CONDUCTIVITY - ROOTS IN CULTURE SOLUTION
K (x½)
NO_3
Br
CONDUCTIVITY - ROOTS IN H_2O
RATE OF CO_2 PRODUCTION (x5)
RATE OF CO_2 PRODUCTION
ABSORPTION PERIOD IN HOURS

Fig. 9. Time effects on accumulation of salt in sap by excised barley root systems.

composition of 0.005 M KBr, 0.005 M KNO_3 and 0.002 M $Ca(NO_3)_2$. Change of solution was made following the 18-hour period, to maintain as far as possible the original concentration. The temperature of the solution was held at 26° ± 1° C.

The CO_2 production was expressed in milligrams of CO_2 produced by each set of root tissues and includes CO_2 evolved and CO_2 from HCO_3 formed in the culture solution.

The points for the 24-hour period are omitted since we have reason to believe that the activity of this set of roots was depressed by some accidental circumstance effective during the initial growth period. The insertion of these points would not modify the general trend of the curves nor the conclusions reached.

Residual solutions and sap expressed from the tissues were analyzed for K, Br, and NO_3. Total sugar content of expressed sap was also determined. Figure 9 presents evidence of significant accumulation of salt within a few hours, with concentrations in the sap rising rapidly during the first 12 to 18 hours. Approximately maximum values for concentrations of K and Br in the expressed sap were attained within this period.

The concentration of NO_3 in the sap increased very rapidly during the period of 16 hours, then fell off abruptly. The absorption of NO_3 continued (as evidenced by the observations on the culture solution, fig. 8), but at the point indicated by the break in the curve, the rate of NO_3 reduction in the tissues greatly exceeded the rate of NO_3 accumulation. The factors controlling reduction of NO_3 need further elucidation, but the suggestion is made that the increasing concentrations of K in the sap influenced the reducing properties of the tissues (ECKERSON, 7). Apparently, unknown factors associated with the seasonal conditions under which plants are grown also modify the ability of the tissues to reduce nitrate. The reduction of nitrate was especially rapid in the experiment now cited. The results on NO_3 absorption emphasize the necessity for examining not only the residual culture solution, but also the tissues, in order to understand the relation of NO_3 absorption to accumulation of NO_3, as such, in the sap. This point is of interest in connection with the study of the relation of oxygen tensions to NO_3 absorption versus NO_3 accumulation.

The significance of data on CO_2 production in relation to sugar loss during a period of salt accumulation is being further investigated, but attention is now directed to the fact that the rate of CO_2 production continued almost undiminished, at least for some time, after the tissues had attained their maximum salt content. The amount of sugar remaining at this point was still relatively large, and adequate for rapid accumulation of salt by low-salt tissues, if we may judge from results of the earlier experiments on the effects of sugar depletion on salt accumulation. The presumption is that any

actively growing cells in the high-salt root system continued to absorb salt after an apparent maximum salt concentration had been attained by the root system as a whole, but this limited absorption would not be determinable from the examination of a mass of root tissues in which most of the cells had attained a high and perhaps maximum salt concentration, particularly if some cells were losing salt, and compensating for gains by actively growing cells.

Further comparisons of salt accumulation by root and storage tissues

The low-salt root tissues had a remarkably high capacity for rapid salt accumulation, greatly surpassing discs of potato tuber in this respect. This difference in behavior can be explained in part by the fact that the potato tuber tissues have a relatively high initial salt content, while root tissues of the kind under consideration are very low in salt for reasons given earlier. The potato tissues, following an initial period of salt loss, continue to absorb salt (KBr) from dilute solution without any approach to a steady state for a period of more than 100 hours (**35**), in contrast to the young root systems which very rapidly attain an approximately maximum average concentration of salt, and in a later period may suffer a net loss of salt.

The writers are of the opinion that the nature of salt accumulation is fundamentally the same for storage tissues and root tissues, but at present it is useful to recognize two aspects of the general problem: (a) the very rapid initial accumulation of salt by low-salt root tissues, and (b) the gradual and sustained salt accumulation by discs of aerated potato tuber tissues. The relatively high-salt concentration characteristic of the latter limits the initial rate of salt accumulation, while, on the other hand, root systems of the type used in our experiments lack the capacity for long sustained salt accumulation because, apart from relatively few cells in the apex, the root cells do not retain the potentiality for constructive metabolism associated with growth. They differ, therefore, in this respect from the surface cells of the discs of potato tuber (**36**). Prevot and Steward (**28**), however, have shown that segments of very young primary roots of barley, relatively high in salt, give time curves for bromide accumulation which have a general similarity to the corresponding curves for bromide accumulation by potato tuber tissues.

One difficulty in comparing salt accumulation by discs of storage tissues and by root systems is that it is not now feasible to assign salt absorption by roots to cells having a particular position with reference to external surfaces. By the use of discs of controlled thickness, Steward *et al.* (**40**) demonstrated that the accumulation of bromide by discs of potato tuber is limited almost entirely to cells lying near the surface, which receive an adequate supply of oxygen and have a very high rate of respiration. Probably the accumulating

cells of these young roots (the cortex is primarily involved) are not limited by the ability of oxygen to diffuse rapidly enough into this relatively shallow zone of cells, which is permeated by intercellular air spaces.

Carbohydrate relations also demand consideration. The potato tuber has a large reserve of carbohydrate available for respiration and other metabolic processes, while the sugar supply of the root tissues is rapidly depleted under conditions of aeration favoring the most rapid accumulation of salt.

The low-salt root tissues were restricted in their initial supply, not only of potassium, but also of nitrogen and phosphorus. Deficiency of either of the latter two elements might limit some phase of metabolism essential to growth and sustained salt absorption over a long period. One might also speculate on the factors associated with shoot growth, apart from carbohydrate synthesis and removal of salt from the root system, which influence the growth activities and salt-accumulating power of the root over extended periods.

Discussion

The relation of metabolic activities of cells to salt accumulation is strikingly illustrated by the experiments on excised root tissues, which afford strong support to the main conclusions of STEWARD, and BERRY and STEWARD, based on experiments with storage tissues, and at the same time present for study certain additional phases of the general question of salt accumulation. A reexamination of many problems of salt absorption and accumulation by roots is demanded from the point of view of cell metabolism and growth. Relations between ions in absorption, concentration relations, nutrient deficiencies, utilization of various forms of nitrogen, etc., together with the control of those variables which determine rates and kinds of metabolism, require further investigation. Oxygen-supplying power of the culture medium, temperature, rate of translocation of carbohydrate from shoot to root, as well as internal factors of cell growth and metabolism, are indispensably concerned with the processes of salt absorption.

Justification is found for emphasizing once more that accumulation of salt by the living cell is not merely a question of permeability. Indeed, many of the methods used in the study of permeability are of such a nature as to preclude the possibility of obtaining data on the absorption and accumulation of solutes by normal metabolizing cells. Emphasis is often placed on the slight permeability of living cells to strong electrolytes. It is now apparent that this statement requires modification with reference to some, if not all, types of plant cells, when initial salt concentration and metabolic activities of the cells are given consideration. Potassium salts can enter the root cell with relatively great rapidity under favorable conditions.

The problem of salt absorption and accumulation by root cells, and also by the living cells of the shoot, is fundamental to the study of the transloca-

tion of mineral elements. These processes also have a direct bearing on certain aspects of root pressure. New points of view with respect to general root metabolism and to certain questions of developmental anatomy arise.

Summary

1. A technique is described for the study of salt accumulation by excised barley roots, which are especially adapted to the investigation of certain general problems of salt accumulation. It is shown that under proper conditions, which are defined, potassium salts accumulate very rapidly in the sap of excised roots, against concentration gradients.

2. A number of variables must be carefully controlled or evaluated in experiments on salt accumulation by excised roots, particularly: (a) age of roots and proportion of actively metabolizing cells, (b) initial salt content of roots, dependent on supply of nutrient salts provided during preliminary growth period, (c) seasonal effects on development of root system, (d) available carbohydrate, (e) variability of material.

3. Accumulation of salt is associated with active aerobic respiration of the roots. An adequate supply of oxygen is indispensable for both cation and anion accumulation. Data pertaining to effects of varying oxygen tensions on salt accumulation are discussed.

4. Temperature coefficients of salt accumulation were found to be of a high order, but evidence of this can be obtained only under experimental conditions carefully chosen to eliminate certain complicating factors.

5. The experiments on excised roots lead to fundamental conclusions similar to those derived from experiments on salt accumulation by storage tissues, and additional aspects of the problem of salt accumulation are presented for study.

6. Attention is directed to the importance of the results on salt accumulation by root cells relative to other problems such as root pressure, translocation of salts, and the general metabolism of root cells.

UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

LITERATURE CITED

1. BARBER, H. H., and KOLTHOFF, I. M. Gravimetric determination of sodium by the uranyl zinc acetate method. Jour. Amer. Chem. Soc. **51**: 3233–3237. 1929.
2. BERRY, W. E., and STEWARD, F. C. The absorption and accumulation of solutes by living plant cells. VI. The absorption of potassium bromide from dilute solutions by tissue from various plant storage organs. Ann. Bot. **48**: 395–410. 1934.

3. BROOKS, S. C. The accumulation of ions in living cells—a non-equilibrium condition. Protoplasma **8**: 389–412. 1929.
4. CANNON, W. A. Absorption of oxygen by roots when the shoot is in darkness or in light. Plant Physiol. **7**: 673–684. 1932.
5. COLLANDER, RUNAR. Permeabilitätsstudien an *Chara ceratophylla*. I. Die normale Zusammensetzung des Zellsaftes. Acta Bot. Fennica **6**: 1–20. 1930.
6. DUNNE, T. C. Plant buffer systems in relation to the absorption of bases by plants. Hilgardia **1**: 208–209. 1932.
7. ECKERSON, SOPHIA H. Conditions affecting nitrate reduction by plants. Contrib. Boyce Thompson Inst. **4**: 119–130. 1932.
8. FURNSTAL, A .H., and JOHNSON, B. Preparation of sintered pyrex glass aerators for use in water culture experiments with plants. Plant Physiol. **11**: 189–194. 1936.
9. HIBBARD, P. L. The chromic acid method for estimation of small amounts of bromine. Ind. & Eng. Chem. **18**: 57–60. 1926.
10. ————, and STOUT, P. R. Estimation of potassium by titration of the cobaltinitrite with potassium permanganate. Jour. Assoc. Off. Agr. Chem. **16**: 137–140. 1933.
11. HOAGLAND, D. R. The accumulation of mineral elements by plant cells. Contrib. Marine Biol. Stanford Univ. Press 131–144. 1930.
12. ————. Mineral nutrition of plants. Ann. Rev. Biochem. **1**: 618–636. 1932.
13. ————. Mineral nutrition of plants. Ann. Rev. Biochem. **2**: 471–484. 1933.
14. ————. The plant as a metabolic unit in the soil-plant system. Univ. California Press. Essays in geobotany. Pp. 219–245. 1936.
15. ————, and DAVIS, A. R. The intake and accumulation of electrolytes by plant cells. Protoplasm **6**: 610–626. 1929.
16. ————, DAVIS, A. R., and HIBBARD, P. L. The influence of light, temperature, and other conditions on the ability of Nitella cells to concentrate halogens in the cell sap. Jour. Gen. Physiol. **6**: 47–62. 1926.
17. ————, ————, and ————. The influence of one ion on the accumulation of another by plant cells with special reference to experiments with *Nitella*. Plant Physiol. **3**: 473–486. 1928.
18. LUNDEGÅRDH, H. Mineral nutrition of plants. Ann. Rev. Biochem. **3**: 485–500. 1934.
19. ————, and BURSTRÖM, H. Atmung und Ionenaufnahme. Planta **18**: 683–699. 1933.

20. ————, and ————. Untersuchungen über die Salzaufnahme der Pflanzen. Quantitative Beziehungen Zwischen Atmung und Anionenaufnahme. Biochem. Zeitschr. **261**: 235–251. 1933.
21. ————, and ————. Zwei verschiedene Atmungsmechanismen in Pflanzlichen Absorptionsorganen. Naturwiss. **22**: 435–436. 1934.
22. ————, and ————. Untersuchungen über die Atmungsvorgänge in Pflanzenwurzeln. Biochem. Zeitschr. **277**: 223–249. 1935.
23. OSTERHOUT, W. J. V. Physiological studies of single plant cells. Biol. Rev. **6**: 369–411. 1931.
24. ————. Permeability in large plant cells and in models. Ergeb. Physiol. exp. Pharm. **35**: 967–1021. 1933.
25. ————. How do electrolytes enter the cell? Proc. Nat. Acad. Sci. **21**: 125–132. 1935.
26. PETRIE, A. K. H. The effect of temperature on the unequal intake of the ions of salts by plants. Australian Jour. Exp. Biol. Med. Sci. **4**: 169–186. 1927.
27. ————. The intake of ions by the plant and its relation to the respiration of the root. Australian Jour. Exp. Biol. Med. Sci. **11**: 25–34. 1933.
28. PREVOT, P., and STEWARD, F. C. Salient features of the root system relative to the problem of salt absorption. Plant Physiol. **11**: 500–526. 1936.
29. PIRSCHLE, K., and MENGDEHL, H. Ionenaufnahme aus Salzlösungen durch die höhere Pflanze. Jahrb. wissen. Bot. **74**: 297–363. 1931.
30. PUCHER, G. W., LEAVENWORTH, C. S., and VICKERY, H. B. Determination of total nitrogen of plant extracts in presence of nitrates. Ind. & Eng. Chem. Anal. Ed. **2**: 191–193. 1930.
31. ROSENFELS, R. S. The absorption and accumulation of potassium bromide by *Elodea* as related to respiration. Protoplasma **23**: 503–519. 1935.
32. STEWARD, F. C. The absorption and accumulation of solutes by living plant cells. I. Experimental conditions which determine salt absorption by storage tissue. Protoplasma **15**: 29–58. 1932.
33. ————. The absorption and accumulation of solutes by living plant cells. II. A technique for the study of respiration and salt absorption in storage tissue under controlled environmental conditions. Protoplasma **15**: 497–516. 1932.
34. ————. The absorption and accumulation of solutes by living plant cells. IV. Surface effects with storage tissue. A quantita-

tive interpretation with respect to respiration and salt absorption. Protoplasma **17**: 436–453. 1932.

35. ————. The absorption and accumulation of solutes by living plant cells. V. Observations upon effects of time, oxygen and salt concentration upon absorption and respiration by storage tissue. Protoplasma **18**: 208–242. 1933.
36. ————. Mineral nutrition of plants. Ann. Rev. Biochem. **4**: 519–544. 1935.
37. ————, and BERRY, W. E. The absorption and accumulation of solutes by living plant cells. VI. The absorption of potassium bromide from dilute solution by tissue from various plant storage organs. Ann. Bot. **48**: 396–410. 1934.
38. ————, and ————. The absorption and accumulation of solutes by living plant cells. VII. The time factor in the respiration and salt absorption of Jerusalem artichoke tissue (*Helianthus tuberosus*) with observations on ionic interchange. Jour. Exp. Biol. **11**: 103–119. 1934.
39. ————, ————, and BROYER, T. C. The absorption and accumulation of solutes by living plant cells. VIII. The effect of oxygen upon respiration and salt accumulation. Ann. Bot. **50**: 1–22. 1936.
40. ————, WRIGHT, R., and BERRY, W. E. The absorption and accumulation of solutes by living plant cells. III. The respiration of cut discs of potato tuber in air and immersed in water, with observations upon surface volume effects and salt accumulation. Protoplasma **16**: 576–611. 1932.
41. VICKERY, H. B., and PUCHER, G. W. Determination of nitrate nitrogen in tobacco. Ind. & Eng. Chem. Anal. Ed. **1**: 121–123. 1929.
42. ZCHEILE, F. P. JR. The thermodynamics of ion concentration by living plant cells. Protoplasma **11**: 481–496. 1930.
43. ZIMMERMAN, P. W. Oxygen requirements for root growth of cuttings in water. Amer. Jour. Bot. **17**: 842–861. 1930.

SALIENT FEATURES OF THE ROOT SYSTEM RELATIVE TO THE PROBLEM OF SALT ABSORPTION

P. Prevot and F. C. Steward

(WITH FOUR FIGURES)

Introduction

The problem examined and the system involved

Investigations on salt accumulation which utilize tissue derived from root systems have a double interest. Not only may they contribute to the elucidation of the mechanism of accumulation regarded as a problem of cell physiology, but also to the further question of the absorption of salts envisaged, not merely in terms of individual cells, but of the root as the most effective of salt absorbing organs. The former aspect has been discussed by Hoagland and Broyer (**10**), and the factors which determine the accumulation of inorganic salts by the cells of barley roots in particular have been outlined. This first discussion concerned only a composite mass of root material which, though accurately reproducible, still comprised cells at all stages of growth and development and of necessity no attempt was made to evaluate their relative efficiency in absorption or to localize along the axis of an individual root those regions most responsible for accumulation. It is the latter aspect of the general problem which is now to be discussed.

The extensive interest (**32**) in the gross morphology and growth of root systems under field conditions is clearly inspired mainly by their more conspicuous rôle of water absorption. In this connection it is generally recognized that the severe delimitation of the absorbing region for water—due to the presence of fat-impregnated tissues (endodermis, exodermis, or even cork) in the older portions of the root system—involves a clear connection between the ability of a root for growth and its efficiency as an absorbing organ. The maintenance of a root surface free from these restrictions is required. A similar general relation must obtain with respect to salts. With particular reference to water, the extent of the absorbing zone has been discussed notably by Popesco[1] (**16**), and, with emphasis upon anatomical and developmental features, by Scott and Priestley (**25**).

We are not here concerned with the extent of the absorbing zone for water apart from the expectation that, at least in so far as mere penetration of the superficial tissues is concerned, the absorbing zone for salts would be as restricted as that for water. Statements to the contrary (**7**), based solely upon the disappearance of solutes from external solutions without examination of the tissues of the root, probably fail to discriminate between the

[1] This paper may be consulted for citations to earlier work.

mere chemical reactions between ions of the solution and the deposits in cell walls or upon external surfaces and actual absorption by living cells of the root. The latter is the major problem. Structural features (**16, 25**) severely limit the absorbing zone for solutes which are unable to penetrate impermeable tissues. It is here that an adequate realization of anatomical features is essential. Since effective utilization by the whole plant demands entry of the salts into the stele, SCOTT and PRIESTLEY (**25**) rightly stress that, as in the case of water so also for salts, the anatomical status of the endodermis—with respect to the distribution of the completely suberized lamellae and passage cells—together with the extent and distribution of a completely suberized exodermis offers the clue to the problem, at least in so far as penetration of the tissues of the root is concerned. Suggestions concerning mere physical penetration may be derived by experiments involving dyes or qualitative color reactions for iron or nitrates, as the earlier workers implied, but it is clear that quantitative data, lacking when the survey referred to was made, are necessary to localize the zones of active salt accumulation. It will be recalled that while SCOTT and PRIESTLEY recognized that cortical cells may accumulate salts, they particularly emphasized the rôle of the endodermis which, they submitted, represents the functional absorbing surface for salts. As a prelude to entry into the stele, this tissue, it was supposed, accumulates the salts from the dilute external solution which, via the cell walls and intercellular spaces,[2] bathes its external surface. At that time there was little evidence (**25**, p. 129) that higher concentrations of a given ion could obtain in the stele than in the outer solution, though a gradient of total concentration, for which salts were not held to be chiefly responsible, seemed to be demanded by the problem of water absorption. It was supposed, therefore, that having accumulated the salts the endodermis lost them again internally. Lacking the recent evidence concerning the more definite effects of oxygen concentration in the salt relations of cells, attention was then directed to the possibility that, by analogy with some evidence drawn from the case of *Nitella*, the higher concentrations of carbon dioxide which may obtain within the stele might be responsible for the loss of solutes internally. Since that time, both with barley and squash, it has been observed repeatedly that the fluid obtained in quantity from within the stele may contain a given solute (KBr) in concentrations much higher than outside the stele (unpublished data of HOAGLAND and BROYER and of STEWARD, see also earlier work of ANDERSSEN, LAINE, SABININ, and STEWARD (**1, 13, 23, 29**). Quite apart from the rôle of cortex and of endo-

[2] The injection of intercellular spaces with liquid (a common feature of the cortex of roots grown in water and responsible for the translucent appearance described by POPESCO) may increase the effective area for diffusion (27) in the cortex, though not in the piliferous layer itself.

dermis respectively in the original absorption, since all solutes which enter the stele must surely traverse the endodermis, SCOTT and PRIESTLEY'S emphasis upon this tissue is to this extent justified. The possibility must be faced, however, that the movement into the stele producing the concentrations observed may be more than a mere passive movement and may itself demand physiological activity on the part of the tissue concerned. Only a quantitative approach can form a basis for the solution of these problems.

Not only upon histological grounds, but also upon the basis of current thought with reference to the mechanism of salt accumulation in cells, it is to be anticipated that the effective region where salts are not merely absorbed, but also accumulated, would be confined to a limited zone at the apex of the root. These views based on evidence derived from *Nitella* (**11**), from storage organs (**28, 29,** and references there cited), and from roots (**10**) have recently been summarized (**5**). Clearly one of the cardinal features of actively accumulating cells is a high metabolic rate maintained by the energy derived from aerobic processes and reflected in the active carbon dioxide production which ensues. This alone, however, may not be effective in salt accumulation if the cells in question have become fully mature, or even senescent, and are unable, by a recrudescence of meristematic activity, to recapture some metabolic process essential for active salt accumulation and apparently characteristic of cells which are rapidly growing and developing (**5**). In short, the normal trend of growth and development, which intervenes between meristematic cell on the one hand and fully expanded, highly vacuolated cell on the other, seems to be associated with a graded intensity of salt accumulation. Cells exhibit this property to the maximum degree when they are likewise at the height of their own active growth and development. This standpoint leads directly to the surmise that the tissues of the root may display a graded activity in salt accumulation determined by the stage of development they have attained. To supply the quantitative data which can alone reveal this fact, many of the experiments here described were made.

HOAGLAND and BROYER (**10**) emphasized the importance of an adequate realization of the nutritional status of the tissue. With tissue of a given age and state of development an initial low salt content is accompanied, under the experimental conditions used, by a high sugar content, and permits a very rapid subsequent absorption of salt. Hence this attempt to assign the effective accumulation to any specific region of the root has been made recognizing that this may be modified by the previous nutrition of the root system, which determines the salt concentration already attained, as well as by the rate of metabolism and by histological features.

Experimental results

Technique and materials

The basic procedure finally adopted resolved itself into the determination of the salt concentration attained, under conditions optimum for accumulation, by root segments the location of which on the axis was definitely known. The segments analyzed were isolated either before or after the absorption period, according to the objective, and a solute not normally present, namely, a bromide (KBr), utilized.[3] The technical difficulties demanded that this study be made only with reference to the bromide ion. However, in view of the evidence available in the papers already cited, the conclusions derived may be extended with safety to include cations.[4]

For practical reasons root segments, free from laterals, were used almost exclusively. An abundance of secondary and adventitious roots, which normally produces an efficient absorbing system and which is a conspicuous feature of the root systems in the condition described (**10**) in the former paper, merely replicates the potentialities of the root apex and for the purposes in question would have proved confusing. Unbranched roots available in large numbers (over 1000 roots were commonly used in a single experiment) and with the maximum length free from laterals were, therefore, desired. Of the roots which never branch, the one tried (hyacinth) involved difficulties apparent with many potential sources of material and will be referred to later. After preliminary examination of roots grown in water culture from bulbs or from the seeds of legumes or squash these plants were finally discarded, either because of the low order of absorption attained, the difficulty of obtaining a uniform population of roots, or troubles due to contamination with fungi. It is now evident that those plants with large fleshy storage organs, such as bulbs or cotyledons, introduce limitations due to the high content of salt already present in the roots, and, in view of the evidence by Hoagland and Broyer (**10**), this may be associated with a relatively low sugar content. As described by Steward (**29**), and Hoagland and Broyer (**10**), these factors combine to reduce the total absorbing capacity, and, therefore, in some cases (*e.g.*, onion), actual accumulation could rarely be detected in the young roots before the leaves had fully emerged. In the first work these low total absorption figures were regarded as inconclusive, but one may recognize here that for the future study of what may be termed "high salt" roots these

[3] The advantages of this solute and the analytical procedure (9) for its determination have been sufficiently emphasized in other writings and need no further reference here. To permit accurate determination of the smaller amounts of bromide special care was taken with the aeration procedure. Freshly standardized 0.001 N thiosulphate—standardized against KBr—was used in a burette, with an appropriate jet, graduated in 0.02 cc. but easily readable to 0.01 cc.

[4] See footnote to page 522.

plants may prove useful. Direct evidence that sugar was at least one factor in the minimal activity of these young roots may be derived from the observation that, both for *Allium cepa* and *Vicia faba,* roots excised from plants, the developing leaves of which had been exposed to light, absorbed more salt per unit weight than similar ones exposed only to darkness (table I). It is worthy of record that the roots derived from plants in the light

TABLE I

EFFECTS OF ILLUMINATION OF THE SHOOT UPON BROMIDE ABSORPTION BY THE ROOT

PLANT	GERMINATION CONDITIONS	FRESH WT. GM. PER 100 ROOTS	DRY WT. GM. PER 100 ROOTS	WATER CONTENT	ABSORPTION PERIOD	Br ABSORBED IN MILLIEQUIVALENTS PER 1000 GM. FRESH WT.
		gm.	*gm.*	%	*hours*	
Allium cepa	Light	2.28	0.093	96	24	5.4
	Dark	1.76	0.068	96	24	4.6
Vicia faba	Light	2.42	0.115	95	24	13.6
	Dark	2.00	0.095	95	24	11.7
	Light	2.35	0.120	95	24	14.4
	Dark	1.94	0.095	95	24	11.4

were consistently greater in fresh weight per unit length of root and seemed to be composed of larger cells.

Of the dicotyledon roots examined, the secondary roots of *Vicia faba* and roots from cotton (*Gossypium*) plants were the most promising material and some confirmatory evidence derived from these plants is quoted. The difficulty of obtaining a constant supply of uniform roots caused us to reject the relatively thick unbranched roots which can be developed on cuttings of woody plants (*e.g., Salix*) in moist air.

The principal experimental material finally chosen was derived from roots of seedling barley plants (*Hordeum vulgare*) of the strain utilized by HOAGLAND and BROYER (**10**) and was restricted to primary roots and those adventitious roots which are always present in the embryo before it germinates and which are identical in structure with the primary root.[5]

[5] It is common knowledge (**2**) that after the development of the first roots from initials present in the embryo of barley, adventitious roots are produced in conjunction with tillers. These are much thicker, differ in anatomical features, and remain unbranched for several inches. The long unbranched roots which were used in this work must be clearly distinguished from these adventitious roots which form later and which were not employed. Reference may be made to BRYANT (**6**) for some anatomical data on barley roots and a description of the effects of aeration. It will be seen by reference to a recent work upon the Gramineae (**2**) that comprehensive studies of the anatomy of the roots of barley as affected by environmental conditions are lacking.

The germination technique outlined in the preceding paper (10) was utilized and then the uniform seedling plants were transferred, when 2 or 3 days old, in a compact layer to a cheese cloth screen above a deep dark tank containing either distilled water or HOAGLAND'S nutrient solution.[6] In this way healthy, long, straight roots free from laterals for distances in excess of 6 cm. could be obtained in large numbers. By controlling the period in distilled water or the nutrient solution roots of the high salt or low salt type could be produced at will. Healthy, actively accumulating roots can be grown even in distilled water[7] for a considerable time (up to about 15 days). If unduly prolonged this treatment affects the efficiency of the apical region; although that of the more mature tissues persists. It is worth emphasizing that the technique used on the roots, by the authors of the preceding paper (10), fostered copious branching, whereas the technique used by the writers tended to suppress the formation of laterals. Granted that the lateral roots virtually duplicate the problem presented by the apices of the unbranched segments, it is clear that the data in this paper permit the probable distribution of the salt absorbed by the whole root system (10) to be described. This was a not inconsiderable factor in the choice of experimental material, which was also influenced by the known adaptability of barley roots to water culture. A further point requires emphasis. Since barley, in common with most of the monocotyledons, is free from secondary growth, the attempt to correlate the absorbing and accumulating regions with the progessive maturation of cells and tissues from the growing region was not complicated either by the early development of cambium in the growing roots or by the incipient formation of a cork meristem in the isolated root segments occasionally utilized. Almost exclusively, excised roots have been used. It has to be recognized that the activity of an excised root of barley is not maintained indefinitely under our conditions. The work of HOAGLAND and BROYER (10), confirmed in the present examination of root segments, revealed that after some hours (approximately 50 to 60 in the present study), a decline, apparently irreversible, in the absorbing capacity occurred. Experiments described in the text show that this point was appreciated in the experiments dealing with the localization of the accumulating region and the major conclusions have been derived from experiments concerned with the period preceding the decline referred to.

[6] $Ca(NO_3)_2$ ..... 0.005 M
KNO_3 ..... 0.005 "
$MgSO_4$ ..... 0.002 "
KH_2PO_4 ..... 0.001 "

[7] The familiar "toxicity of distilled water" is not here in question. Water of the exceptional purity to possess these toxic properties would not retain them in contact with so large a quantity of living tissue.

The only other technical details concern the isolation of the root segments and the conditions adopted for absorption. In all cases the small root segments were cut transversely[8] by hand with a sharp safety razor blade from small batches of roots laid parallel, in a single layer, with their apices alongside. Previously, two brief washings with distilled water removed surface contamination from the bromide solution, and those roots awaiting cutting did so in distilled water. After cutting, the roots were surface dried with drying paper, and subsequently both the fresh and dry weights of the samples were recorded. These operations need considerable care to avoid unnecessary manipulation, or actual loss of sap, but the reproducibility of the blotting technique may be recognized from the estimation that the error in the fresh and dry weights of comparable samples did not exceed 5 per cent. The somewhat greater error in cutting the segments is mainly eliminated by expressing the salt content per segment, not in absolute terms, but per unit fresh weight. The ashing procedure demanded by the bromine determination was done in an electric furnace in the presence of caustic soda.[9]

The bromide content of the roots is expressed as milliequivalents per 1000 gm. fresh weight. Since the water content of the roots is high (over 90 per cent.), this may be compared directly as a concentration with that of the external solution. Only very special purposes and precision greater than that necessary in this paper would demand the calculation of concentrations of absorbed salt in terms of the actual water content of the tissue. The absorption experiments were conducted by growing the roots in a 0.005 M KBr solution with a rapid stream of air in excess of that actually demanded by the needs of the tissue. Both the aeration vessels described by ROSENFELS (**22**) and jars containing coiled perforated block tin aerators have been used. Respiration determinations have not been made in this first part of the problem, though they will be clearly desirable in the future.

THE EXTENT OF THE ABSORBING SURFACE

Examination confirmed that the roots of barley grown in water culture had no suberized superficial tissues even at the level where laterals appeared. All the cells external to the stele were therefore in free contact with the external solution. Some experiments were made to verify the perhaps obvious presumption that salt could enter the superficial tissues over the whole surface of the root. In these experiments the question of accumulation after

[8] No attempt was made to determine the radial distribution of the most actively accumulating regions. For some reference to this problem on the basis of qualitative experiments, see POPESCO (**16**, figs. 22–25).

[9] Caustic soda is preferable to sodium peroxide, especially where dry tissue is concerned.

TABLE II

COMPARISON OF THE BROMIDE CONTENT OF ROOT SEGMENTS CUT BEFORE AND AFTER ABSORPTION*

PLANTS	SEGMENTS CUT BEFORE ABSORPTION		SEGMENTS CUT AFTER ABSORPTION	
	REGION (0 = APEX)	Br MILLIEQUIVALENTS PER 1000 GM. FRESH WT.	REGION (0 = APEX)	Br MILLIEQUIVALENTS PER 1000 GM. FRESH WT.
Barley 6 days old	0 to 4 cm.	33.4	0 to 4 cm.	29.7
	4 cm. to end	30.0	4 cm. to end	32.9
Barley 14 days old	0 to 3 cm.	27.5†	0 to 3 cm.	30.1
	3 to 6 cm.	32.7	3 to 6 cm.	30.0
	6 to 9 cm.	30.8	6 to 9 cm.	31.1

* Absorption period: 15 hours in 0.005 M KBr solution.

† There was some apparent injury to the tissue in this case.

penetration was not a principal concern and hence the root segments analyzed were comparatively large—too large, in fact, to reveal the longitudinal gradients of accumulation to be discussed in a subsequent section. To facilitate the analytical procedure "low-salt" roots grown in distilled water and capable of marked salt accumulation were used.

The three types of procedure used were as follows:

1. Segments were cut from the barley roots *before* they were immersed in 0.005 M KBr solution, and after an appropriate period in this solution they were analyzed for bromide. In this case the salt might penetrate via the cut ends (*e.g.*, by the vessels or large, central, vessel-like cavity) or the external piliferous layer. The contribution of the transverse surfaces exposed, in comparison to the total surface, was small.

2. Similar roots were immersed for an identical period but in this case the entire length free from laterals remained intact. Subsequently they were subdivided into lengths identical with those in the preceding treatment and analyzed for bromide. This comparison was made for two separate series which involved plants of different age, namely, 6 days and 14 days (table II). It will be evident from the tables that all the root segments absorbed bromide, and equally so, whether they were present in the intact root or were isolated from it previously. This result strongly suggests that the effect of transverse cuts is negligible and that, granted contact with the bromide solution via the external surface, this solute can penetrate to all portions of the root which can absorb it and that no advantage accrues from contact of the older segments with the younger, or from the direct contact

of cortical cells, not bounded by a piliferous layer, with the external solution. The absence of intercellular spaces injected with liquid in the latter tissue does not therefore restrict penetration appreciably.

3. As a further proof that bromide penetrates freely through the external surface of the root and that neither the cut ends of the root nor the apex are more efficient for penetration than the intervening region, the third procedure, which exposed only a median zone leaving the proximal and distal ends out of direct contact with the bromide solution, was adopted. Long unbranched roots (6 cm.) were bent into a loop, their cut ends and apices penetrating a thin agar plate, (fig. 1) which prevented contact between

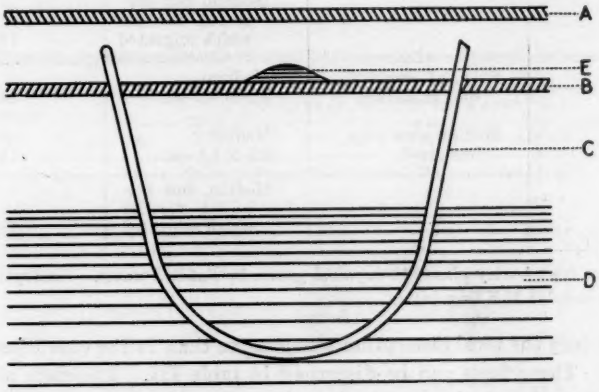

FIG. 1. Illustration of method of studying the surface available for absorption by roots (see table III): *A*, glass plate; *B*, agar plate; *C*, root; *D*, potassium bromide solution; *E*, drop of water.

the apex or cut end and the bromide solution. The bromide content was determined separately upon the median portion in direct contact with the solution (1.5 to 4.5 cm. from apex) and the combined proximal and distal remnants. Comparison was then made with control root pieces entirely immersed in the usual way and subsequently cut into segments of the same size. In the roots treated by the procedure of figure 1 some bromide migrated into the proximal and distal portions which were not in direct contact with the bromide solution. It can be shown that if this bromide had remained in the median segment it would have had bromide content almost identical with that of the roots completely immersed. In other words, as much bromide passed through this portion of the root surface by one procedure as by the other. In the roots totally immersed, the available surface for bromide penetration was the whole root surface (the volume for accumulation the same in both cases),

TABLE III

THE ROOT SURFACE AVAILABLE FOR SALT ABSORPTION

EXPERIMENT	ABSORPTION* CONDITIONS	ROOT SEGMENT	Br ABSORBED IN MILLIEQUIVALENTS PER 1000 GM. FRESH WT.
A	6 cm. of primary root immersed	Median 1.5 to 4.5 cm.	18.8
	Median zone only immersed	Median 1.5 to 4.5 cm.	11.2
		Median, but corrected for Br which migrated	17.2
B	6 cm. of primary root immersed	Median 1.5 to 4.5 cm.	24.0
	Median zone only immersed	Median 1.5 to 4.5 cm.	16.8
		Median, but corrected for Br which migrated	22.9

* Roots from barley plants 12 days old grown in distilled water. Absorption period: 16.3 hours in 0.005 M KBr solution.

and therefore the total absorption was greater than in the case illustrated in figure 1. These facts can be discerned in table III. The data also imply that in these detached roots the bromide can reach the accumulating cells more readily by direct penetration of the external surface than it can by longitudinal migration from bromide solutions applied at other points along the root axis. The absorbed bromide is mainly in the cortex and if the movement which does occur is restricted to that unspecialized tissue this result would be intelligible.

These results can be summarized by saying that for barley roots grown in water culture the whole root surface between the apex and the emergence of secondary roots represents a potential absorbing surface for salts. This is in harmony with the complete absence of suberized superficial tissues. Data are not available for unbranched roots *with* a suberized exodermis. In roots previously deprived of salts, bromide can be accumulated even at distances exceeding 5 cm. from the apex at which level, in *these "low-salt" roots especially,* the endodermis is completely suberized and has much thickened radial and inner tangential walls. Whether the salt accumulated by the cortical cells at this level can be available for the tissues within the stele is an open question.

Distribution of accumulation in roots

The aspect of the problem which involves the distribution of accumulation in roots is in many respects the most important one. It follows that in the immersed roots just discussed the bromide solution can penetrate freely to the accumulating cells, and that any differences of concentration attained along the longitudinal axis reflect the capacity for accumulation in the cells concerned, and that the factor of penetration of the superficial tissues need not be considered further.

The data of tables IV, V, VI, VII, and VIII comprise the results of absorption experiments in which the roots were cut into segments at the end

TABLE IV

Distribution of absorbed bromide in segments of roots* from "low-salt" plants

Absorption period	Distance of segment from apex					
	0–1 cm.	1–2 cm.	2–3 cm.	3–4 cm.	4–5 cm.	5 cm.–end
hours	Br in milliequivalents per 1000 gm. fresh weight					
5	14.2	.....	13.2	11.3	.....	9.2
10	31.8	23.6	16.4	13.2	13.8	14.6
24	49.5	40.8	34.5	33.4	29.5	30.2
30.3	51.5	47.8	37.9	33.9	32.6	26.4†
47	62.8	59.2	50.5	37.4	.....	.....
101	49.2	22.6	14.3	16.1	24.7	24.0

* Barley roots from plants 9 days old grown in distilled water. External solution for absorption period: 0.005 M KBr.

† Value open to question.

TABLE V

Distribution of absorbed bromide in segments of roots* from "high-salt" plants

Absorption period	Distance of segment from apex			
	0–1 cm.	1–3 cm.	3–5 cm.	5 cm.–end
hours	Br in milliequivalents per 1000 gm. fresh weight			
9	15.8	9.65	7.85	.....
23	20.0	13.90	12.50	13.6
50.5	27.1	22.70	19.10	.....
79	29.3	22.90	17.70	.....

* Barley roots from plants grown 15 days in nutrient solution. External solution for absorption period: 0.005 M KBr.

TABLE VI

DISTRIBUTION OF ABSORBED BROMIDE IN SEGMENTS OF ROOTS* OF LOW ABSORBING CAPACITY

ABSORPTION PERIOD	DISTANCE OF SEGMENT FROM APEX				
	0–1 CM.	1–2 CM.	2–3 CM.	3–4 CM.	4–5 CM.
hours	Br in milliequivalents per 1000 gm. fresh weight				
10	4.27	3.78	1.91	1.34	.....
65.3	14.4	10.2	7.92	2.79	2.62

* Barley roots from plants grown in half-strength nutrient solution. External solution for absorption period: 0.005 M KBr.

TABLE VII

DISTRIBUTION OF BROMIDE IN DIFFERENT SEGMENTS OF ROOTS* OF *VICIA FABA*

ABSORPTION PERIOD	DISTANCE OF SEGMENTS FROM APEX		
	0–2 CM.	2–4 CM.	4–6 CM.
hours	Br in milliequivalents per 1000 gm. fresh weight		
24	10.5	7.45	7.10

* Roots from plants grown in sand irrigated with distilled water.

TABLE VIII

DISTRIBUTION OF BROMIDE IN DIFFERENT SEGMENTS OF ROOTS* OF *GOSSYPIUM* SP.

ABSORPTION PERIOD	DISTANCE OF SEGMENTS FROM APEX				
	0–1 CM.	1–2 CM.	2–3 CM.	3–4 CM.	4 CM.–END
hours	Br in milliequivalents per 1000 gm. fresh weight				
20.3	22.0	12.1	7.75	7.35	7.35

* Roots from plants grown in nutrient solution. External solution for absorption period: 0.005 M KBr.

of the absorption period. It will be observed that the longitudinal distribution of accumulation has been assessed for plants with both the "high-salt" and "low-salt" type of nutrition, referred to previously, and also at various times after the roots were excised and introduced to the absorption conditions.

In table IV, for plants of the "low-salt" type, which ultimately absorb bromide to high concentrations, a pronounced longitudinal gradient of accumulation along the axis of the root is evident (fig. 2[10]). The highest

[10] Note that in the figure the sap concentrations are plotted at the mid-point of the segment concerned. The curves were drawn to give a smoothed fit.

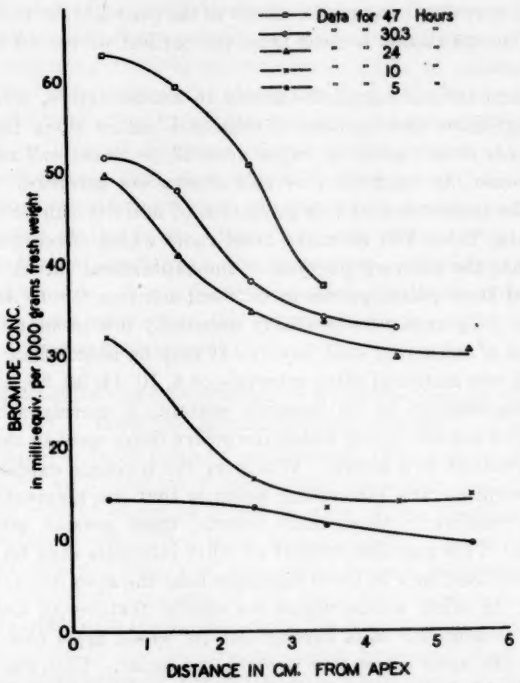

FIG. 2. Distribution of bromide in primary roots. (See table IV).

concentrations were attained in the samples nearest the apex (their mid-point at 0.5 cm. from the tip), and they decrease progressively and consistently[11] to the oldest tissue examined. It should be stressed that this effect is discernable at the earliest periods when samples were taken, and it persists consistently throughout. Subsequent to 47 hours and prior to 101 hours, the onset of the decline mentioned earlier in this paper causes actual decrease in the bromide content of the tissue samples, and not till then do irregularities appear in the series.[12]

By contrast with table IV, the data of table V refer to roots of the "high-salt" type. This explains the lower concentrations of bromide which were attained. To compensate for this, larger tissue samples were necessary, and, to avoid a too unwieldy total number of roots, these had to be obtained by

[11] Irregularities arise only when the apical region is definitely injured by nutritional or other causes.

[12] Examination of the roots before and after the experiment revealed that a progressive suberization of the endodermis occurred during the experiment so that the stage definitely recognizable at 4 cm. from the tip before the absorption period had encroached to 0.5 cm. by the end of it.

increasing the size of the segments. Even so the evidence for the longitudinal gradation of accumulation is clear from the earliest period (9 hours) to the latest (79 hours).

It is evident that the graded activity in accumulation, which coincides with the progressive development of cells and tissues along the axis of the root, *surmounts those factors of sugar content, previous salt nutrition, etc., which determine the total level of salt absorption attained.* An extreme example of the persistence of this gradation of activity can be derived from one set of data (table VI) obtained from roots which effected so little total absorption that the primary purpose of the experiment failed. These roots were obtained from plants grown in nutrient solution for 15 days and were excised when the plants were probably unusually low in sugar (4 A.M., and after a period of relatively dull days). It may be noted that, although the tissue (which was analyzed after intervals of 4, 10, 14, 30, 58, and 65 hours) increased progressively in its bromide content, it merely attained, in 65 hours, a level of concentration which the active tissue used in the experiment of table IV reached in 5 hours. Whatever the ultimate explanation of the low total absorption, the interesting point is that the segments nearest the apex retain, relative to those more remote, their greater activity in salt accumulation. This was also evident at other intervals than 65 hours and it should be noted that only in those segments near the apex did actual accumulation occur. In other words, where the special features of the root do not permit it to accumulate salts throughout its whole mass *this may still be possible near the apex where new growth can occur.* This was also evident in those earlier experiments described with roots from other plants in which only low total absorption was attained. The figures (table VII) obtained with the secondary roots of *Vicia faba* illustrate this point. Of particular interest concerning the gradation of accumulation normally operative in roots growing under standard conditions of water culture with aerated solutions are the data supplied for cotton plants (table VIII). The roots were obtained from plants growing successfully in the greenhouse for other purposes, and they again revealed a similar longitudinal gradation of salt accumulation—albeit associated with the relatively low degree of accumulation which would now be anticipated from the high level of nutrition they received during growth.

The fact of the longitudinal gradation of accumulation[13] is then established as a general property of roots, and this persists concomitant with either a high or a low total capacity for salt absorption determined by other variables.

[13] Since the completion of the work here reported Mr. J. A. HARRISON of Birkbeck College, University of London, has collaborated with the authors. By the application of a quantitative spectroscope technique it has been possible to demonstrate the longitudinal gradation of accumulation of potassium in barley roots, and also of rubidium absorbed during experiments similar to those in this paper.

Figure 3 correlates the principal anatomical features of barley roots grown in water with their activity in salt accumulation. The horizontal histograms represent at three selected levels (data in tables IV and V at 24 and 23 hours respectively) the absorption by roots of both the "high-salt" and "low-salt" type. The distribution of stele and cortex at these levels may be discerned in the scale diagrams shown[14] and the status of cortex (nos. 1, 2, and 3) and of stele (nos. 4, 5, 6, and 7) is apparent from the scale diagrams, which were obtained from camera lucida drawings at the magnification indicated on the figure. Root hairs are omitted from the diagrams, although they were plentiful on the roots used—especially those grown in full nutrient solution. For a further discussion of these structural features and for some data relative to the longitudinal plane, the section headed histological evidence may be consulted.

Interrelations in the effects of nutrition, sugar content, time, and developmental factors

From the paper by Hoagland and Broyer (**10**) it is clear that roots of the "high-salt" and "low-salt" types differ greatly in their sugar content. The probable rôle of sugar as a determinant of the observed longitudinal gradation of salt accumulation must be considered. Direct evidence of the efficacy of an external supply of sugar may be seen in figure 3, which also serves to re-emphasize the form of the absorption/time curves usually obtained in this work with the excised roots (fig. 4 and tables IV and V). With a slight lag, probably due to the time taken for sugar itself to penetrate, the low total absorption of the "high-salt," low-sugar roots used in this experiment was raised almost exactly to that of the "low-salt" roots by an external sugar (glucose) concentration of 2 per cent. This suggested that an external sugar supply might raise the absorption obtained by the roots of bean, onion, etc., to the order of that of the sugar-rich, "low-salt" barley roots. Although 2 per cent. glucose in the external solution raised the absorption from 8.75 to 12.3 milliequivalents per 1000 gm. fresh weight in secondary roots of *Vicia faba,* the high values characteristic of the barley plants referred to were not realized. From the data of figure 4 it is also clear that the factors responsible for the ultimate decline of excised barley roots are not such that they can be corrected by an external supply of sugar. On the contrary, there is evidence that, despite their low sugar content, the "high-salt" roots are able to continue absorption (albeit at the low level characteristic of them) for a still longer period.[15] The onset of this decline, which is accentuated by the extensive sugar metabolism (**10**) in the "low-

[14] Note that the longitudinal and transverse scales are different.

[15] Since the data for potassium are lacking it is not possible to state how much of the bromide absorbed was accompanied by potassium. Data for potassium content would have given precision to the term "high-salt" roots and permitted a closer comparison between these data and those in the earlier paper than is now possible.

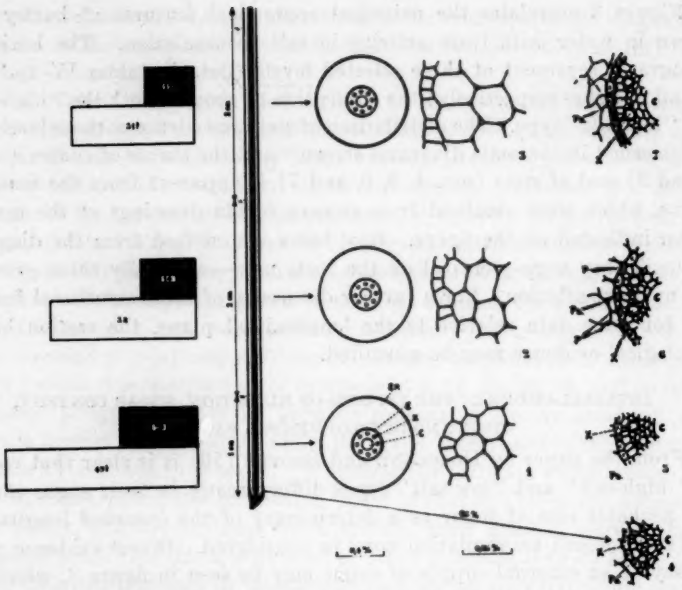

FIG. 3. Right, anatomy of barley root ("low-salt" type), at different distances from apex, in relation to bromide accumulation. Nos. 1, 2, 3, cortex and piliferous layer without suberized exodermis. No. 4, 0.6 mm. from root apex. Differentiation of vascular strands quite evident. No. 5, 5 mm. from root apex. Unsuberized endodermis. No. 6, 1 to 3 cm. from root apex. Endodermis suberized but with passage cells opposite xylem groups. No. 7, 3 to 5 cm. from root apex. Endodermis suberized, without passage cells, and, at extremity of segment, also thickened. *Ep*, piliferous layer; *E*, endodermis; *X*, xylem; *Ph*, phloem; *P*, passage cell; *C*, central cavity (axile vessel).

Left, ▭ "low-salt" roots: bromide absorbed by segments; data of table IV;
▬ "high-salt" roots: bromide absorbed by segments; data of table V.

salt" roots and appears with some consistency at approximately 60 hours with barley, must be conditioned by some factor *peculiar to excised roots*, and until this can be completely specified it must suffice to contrast it with the maintained ability for absorption of attached roots—a property which must surely be identified with their continued growth as well as with the translocation of salt from root to shoot referred to by HOAGLAND and BROYER (10). It is not unreasonable that the "low-salt" excised roots from plants on the verge of actual deficiency symptoms would be the more quickly limited in regard to continued growth than those of the "high-salt" type.

At this point a comparison[16] with the form of other similar curves may

[16] The root experiments in this section were made by withdrawing random samples from a composite batch of root material at the times stated. They were not made only on uniform roots free from laterals as heretofore.

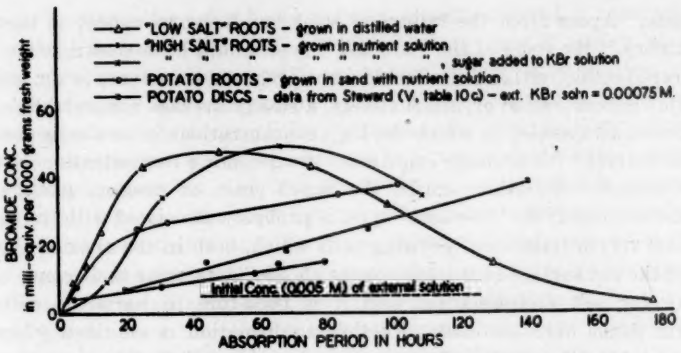
"LOW SALT" ROOTS – grown in distilled water
"HIGH SALT" ROOTS – grown in nutrient solution
" " " " " " " " , sugar added to KBr solution
POTATO ROOTS – grown in sand with nutrient solution
POTATO DISCS – data from Steward (V, table 10c) – ext. KBr soln = 0.00075 M.
BROMIDE CONC. in milli-equiv. per 1000 grams fresh weight
0
20
40
60
Initial Conc. (0.005 M) of external solution
0
20
40
60
80
100
120
140
160
180
ABSORPTION PERIOD IN HOURS

FIG. 4. The effect of time on bromide accumulation-results for tissues.

be profitable. In figure 4 data for potato discs in much more dilute solution (0.00075 M KBr) are plotted (**28**, p. 230). The potato discs are clearly constituted of cells already high in salt, and which maintain their supply of soluble carbohydrate from starch hydrolysis, and are known to be capable of a renewed and protracted activity in growth which eventually (and especially if the tissue is in moist air) culminates in cell division. The slope and general similarity between this curve and that for barley roots of the "high-salt" type requires little comment. Surely both reflect the maintained and progressive activity of the constituent cells at a level fixed alike by their sugar and initial salt content, oxygen supply, and their ability to continue the processes of growth. The similar curve, also shown in figure 4 for potato roots from plants grown in sand culture, suggests two distinct phases. In the first phase (complete by 24 hours) a rapid increase of bromide concentration is reminiscent, though less striking because the nutrient conditions were not so extreme, of the "low-salt" barley roots. The implication is that in both cases (potato roots and "low-salt" barley roots), during the first phase of contact with the potassium bromide, the "low-salt" condition in the initial tissue (now known to be associated with high sugar content) permits a more rapid salt entry than would otherwise obtain. In this first phase, which is really determined by the conditions during previous development, further growth and development of the root cells probably plays but a minor rôle. The second phase is clearly homologous with the behavior in time of the tuber tissue, and of the "high-salt" barley roots and must surely be identified with the slow but maintained processes of growth at least until over 100 hours. During this period the *average concentration* of the reference element (bromide) *in the whole tissue system*[17] increases steadily as successive cells mature, thus increasing the proportion of tissues which have passed through their most active periods of salt accumulation in the presence of

[17] This must be clearly distinguished from the total content of salt in the root system.

bromide. Apart from the failure of some fundamental aspect of the vital machinery,[18] the roots of the "low-salt" type would have shown, after their first rapid intake, either a similar slow but progressive increase in the average bromide concentration or, alternatively, a steady average concentration (the maximum attainable) in which the high concentrations in new cells developing in contact with bromide counteract the declining concentration of those more mature. In other words, the rapid onset of decline, particularly evident in roots of the "low-salt" type, is probably associated with the failure of those very attributes of growing cells which, both in the apex of the root and at the cut surface of storage tissues (**5, 29**), determine their conspicuous ability for salt accumulation, and it is therefore in harmony with the general thesis here outlined. Further confirmation is obtained when the data of table IV are plotted as time curves when it becomes clear that the apical segments, where the capacity for growth lingers longest, retain their salts until somewhat after the onset of decline in the older tissues.

Since excised roots can remain capable of protracted growth, and presumably of salt accumulation, for almost indefinite periods in sterile nutrient solutions, or on agar, containing sugar and yeast extract (**20, 21, 33, 34**), the question arises whether yeast extract would have any effect upon salt accumulation by excised roots of barley and particularly their apical segments. Briefly, in these relatively short experiments, no effects on salt accumulation due to yeast extract have yet been observed which could not be ascribed to its sugar content.[19]

Experiments were done to ascertain the effect of sugar upon the longitudinal gradation of accumulation. These admittedly suffered from the use of too large individual segments (4 cm.) and are not given in detail. Although positive effects due to sugar were obtained (6-day-old plants), they did not alter the relative efficiency of the different segments (0 to 4 cm., and 4 to 9 cm.), but, instead, raised the capacity for absorption of the root as a whole. Indeed the very fact that the longitudinal gradation of accumulation is most pronounced when the roots have highest sugar content and show least response to an external sugar supply ("low-salt" roots) eliminates the possibility that this gradation is due merely to a similar gradient of sugar concentration in the cells.

Distribution of metabolic activity

One further clue to the metabolic basis for the observed gradation of accumulation in the root can be derived from the fact that the regions of most active accumulation apparently exhibit the greatest avidity for oxygen.

[18] This condition is not to be confused with death since the cells in question may still produce carbon dioxide (10) and complete loss of solutes only appears at a later stage of decline.

[19] No reflection upon the validity of White's work (33, 34) is here intended.

This may be observed readily by an indirect procedure based on observations of LUND and KENYON (**14**). The apical region of roots of barley, as used in this work, stains deeply with methylene blue,[20] whereas the older tissues stain lightly and more superficially. The deep staining of the apical region responds to external conditions, but not so the superficial stain further back. In oxygen-free water, in nitrogen or a vacuum, the apical segment rapidly becomes colorless, and transfer to air proves that this is reversible. In heat-killed tissue, on the other hand, the deeply stained apex is unaffected by an anaerobic environment. These simple tests strongly suggest that the apical segment is a center of more intense oxidation (reducing the dyestuff under anaerobic conditions) than the older tissues. In view of the emphasis recently placed upon the relation between accumulation and *aerobic* metabolic processes (**28, 29, 30**) this has particular significance. So much so that confirmation by direct oxygen measurements, not yet available, would be of value.[21] LUND (**14**) and his coworkers believe that electrical measurements which can apparently be correlated with oxidation also indicate the gradation of metabolic activity along the axis of the root.

HISTOLOGICAL EVIDENCE

The previous paragraph offers tentative but direct evidence in support of the presumption that the region of greatest activity in accumulation is also conspicuous for its activity in metabolism. However, histological evidence is adequate in support of this position.

Practical considerations prevented the use of root segments shorter than 1 cm. Even so, satisfactory analytical data demanded very large numbers of roots. It will be recalled that even a distance of 1 cm. behind the apex includes not only the meristem but also the first and the most conspicuous stages of cell extension. Current views no longer restrict entirely the cell divisions to the apical meristem (**18**) but recognize that cells which have already enlarged and become conspicuously vacuolated may still remain capable of division, though they do so only infrequently. The apical segment, therefore, where the activity in accumulation is most intense, comprises a limited zone of conspicuous cell division but is mainly composed of cells in which growth by extension predominates over division and which, for present purposes, may be described as at the height of their growth and

[20] Acknowledgment is made to Dr. M. M. BROOKS for a supply of pure methylene blue.

[21] HENDERSON (8), who reported data for oxygen consumption in conjunction with water absorption, did not distinguish between different portions of the root surface.

[22] It is apparent that the need for an even more intimate examination of the apical segment suggests itself. This, however, must await a technique, possibly based on the rapidly developing methods of micro-incineration, which can yield with certainty quantitative data for individual cells so that their activity in accumulation may be more closely related to their development. The more qualitative observations of PENSTON (15) relative to potassium are suggestive in this connection.

development. The rapid histological changes which occur therein are perhaps the best testimony to their metabolic activity.[22]

The great activity of the apical segment is therefore to be identified not with the apical meristem, but rather with cells already possessed of large aqueous vacuoles and in which the evident ability for further extension and division prompted the term "vacuolating dividing cells" applied by PRIESTLEY (18). It is an interesting, but as yet an open question, whether the vacuole, in the form in which it may now be revealed by special cytological methods in meristematic cells, can also be associated with salt accumulation. Neither the staining experiments of POPESCO (which no doubt reflect in the apex of the root the distribution of large vacuoles rather than the intrinsic ability of cells to accumulate), nor the impermeable properties of the root apex inferred by PRIESTLEY and TUPPER-CAREY (19) (on the basis of its resistance to flow and to alternating electrical current) are necessarily incompatible with the ability of the meristem to accumulate salts. POPESCO's experiments, which excluded the meristem from the absorbing region, did, it is claimed, reveal the most intense absorption *near its low limit*[23] (16, pp. 56, 57, and 130). However, as inferred elsewhere, the detailed treatment of the meristematic region is outside the scope of the quantitative methods at present available, but since it occupies so small a proportion of the apical segments used, the quantity of salt it could contain would have little effect upon the quantitative comparisons reported in this paper.

The following data in table IX were derived from actual measurements

TABLE IX

DIMENSIONS OF CELLS AT DIFFERENT DISTANCES FROM THE ROOT APEX OF BARLEY

Distance from apex (mm.)	0.24	0.6	1.2	2.4	7.4	35.0
Mean length per cell (μ)	11.0	17.4	42.6	46.5	150.0	198.0
Mean diameter per cell (μ)	20.3	26.1	15.6	19.2	33.0	22.8

of cell sizes from roots, as used in these experiments, which were embedded in paraffin and examined in serial section for both transverse and longitudinal planes. The figures serve to stress the location on the axis of the most conspicuous enlargement of cells. At levels far enough from the apex for stele and cortex to be distinguishable, they were measured separately. Since the proportion of cortex to stele (even including the central cavity or axile vessel) in the roots is high, of the order of 9 : 1 (fig. 3), the accumulation data are reflected mainly by the cortex and therefore the

[23] POPESCO (16, p. 57) says: "Le maximum de pénétration du nitrate de potassium, dans la racine de *Soja hispida*, à lieu entre 1 et 4 mm. de la pointe, où il n'y a pas de poils radicaux. A partir de ce point, la pénétration du sel dans la racine diminue progressivement, pour s'arrêter presque complètement à partir de 10 mm.: à ce niveau l'endoderme parait brun."

figures for the stele are omitted. As would be supposed, the major dimensional changes are in the longitudinal direction and it is interesting that even at 7.4 mm. behind the root apex the cortical cells have already attained a length over 10 times that of the original meristematic cells. Further extension, of course, continues (compare the data at 35 mm. from the tip) though necessarily more slowly. Unfortunately, the examination of these roots did not permit a definite statement concerning the maximum distance from the apex at which sporadic divisions, and consequent enlargement of daughter cells, could occur in the root parenchyma. That it would be unwise to eliminate this factor even at considerable distances from the apex is evident by analogy with the mesophyll, where divisions persist until comparatively late in the ontogeny of the leaf (**4**). AVERY (**3**) records that no cell divisions occur during the development of the cortex of primary roots of *Nicotiana* in the transverse plane, but apparently did not examine longitudinal sections from this point of view. In the examination of roots used in this paper, the frequent occurrence of short parenchyma cells suggests that transverse divisions occur relatively far from the apex. According to WAGNER (**31**, p. 8), who has determined the distribution of mitoses in the root apex of *Allium*, the depth of the zone where mitoses occur abundantly is about 1100 to 1850 μ. It is clear also that cells lose their capacity for division neither suddenly nor completely, but that in the case of *Allium* the proportion of cells in mitosis at a given time has declined to less than 1 per cent. of its maximum at a level where the mean cell length is 5 times that of the very short cells of the tip (**31**, fig. 11, p. 27). The maximum distance from the tip at which division can occur is, however, still uncertain.

The basis, therefore, for the longitudinal gradation of accumulation is to be sought in factors which determine the nutrition, growth, and development of *cortical cells*. The demarcation of the functional absorbing zone, by reference to stages of vascular differentiation and of the development of the endodermis (**24, 25**), utilizes an indirect relation which is not a causal one, and which is of value only in so far as these more easily recognized structural features reflect the condition of the cortical cells which are more directly concerned with salt accumulation. With respect to the problem of the translocation, rather than the absorption of salts, the importance of the histology of the endodermis still remains, since the solutes must surely enter the stele where this tissue is not completely suberized. However, full discussion of the factors which govern the removal of salts from the root is outside the scope of this paper.

The results of this perhaps too cursory anatomical treatment are therefore in accord with the view that the relative efficiency of the tissues nearest the root apex must be ascribed to the preponderance of active cells rapidly enlarging and even dividing. No doubt it will be possible eventually to specify the metabolic significance of these general histological comparisons. At present, it is possible only to note the implied and suggestive parallelism

(and the evidence does not warrant any stronger statement yet) between the activity of the cells of the root in protoplasmic (protein) synthesis and their corresponding activity in salt accumulation. Previous investigations with storage tissues, though concerned with tissue tending to re-approach rather than recede from the condition of active cell division, have also shown that salt accumulation is most intensely exemplified by those cells whose vital activity is such that it would normally culminate in division. A broad survey (**29**) of the accumulation displayed by many types of cells and tissues reveals that as these vital activities decline so also does the capacity for salt accumulation and especially so with respect to the absorption of ions of both signs which has been designated "primary absorption." With this point of view the present evidence of the longitudinal distribution of accumulation on the axis of the root is in full accord. The consistent fact which emerges is that cells which are capable of most active growth, which are at the peak of their own vacuolation and extension, attain the greatest degree of salt accumulation. Though external factors may control the total absorption obtained, this does not counterbalance that graded activity which is an inevitable consequence of factors operating in the progressive development and maturation of cells from the root apex.

RÔLE OF ROOT CORTEX

The survey of the root as an absorbing organ, to which earlier reference was made (SCOTT and PRIESTLEY, **25**), implies that under the conditions which obtain in water culture the rôle of cortical cells, and of root hairs when they are present, would be secondary. When, as in the earlier account cited, the endodermis is endowed with the ability both to accumulate salts and to release them to the stele, attention may be focused upon this tissue to the exclusion of the cortex—the probable rôle of which may be thereby unduly restricted. The very technique which produces active "low-salt" roots testifies to the ready removal of solutes from these cortical cell to the growing plant. This possibility was rejected by PRIESTLEY and this led to the emphasis upon the endodermis as the functional absorbing surface. It is clear that in the experiments of HOAGLAND and BROYER (**10**), as in ours, the bulk of the solute absorbed and accumulated was present in cortical cells which, there is every reason to suppose (see section on the extent of the absorbing surface), absorbed it directly from the external solution. The results here presented suggest that the "low-salt, high-sugar" type of root contains cortical cells capable of accumulation even at levels where the endodermis may commonly be suberized and even free from passage cells. Though affected somewhat by nutrition, the endodermis of barley at 3 cm. from the root apex is commonly suberized, but usually has passage cells which have disappeared completely at 5 cm. An interesting problem is here presented as to whether or not the salt content of the cortex at this level can be withdrawn by the more active regions

and subsequently enter the stele. Though the experiment described by PREVOT (17) emphasizes how readily *attached* roots which have accumulated bromide can subsequently lose it when the conditions for removal and translocation obtain,[24] even at levels some distance from the apex (4 to 5 cm.), it does not establish conclusively that bromide can move out of the older cortical cells to the stele across an endodermis which is definitely and completely suberized. In short, the cells of the root cortex of *attached* roots function for a considerable period after their formation for the temporary storage of inorganic salts and remain capable, reversibly, of accumulation from the external solution and rapid loss[25] to the presumably more active tissues of the growing shoot. Clearly, the contribution of the root cortex to the functional absorbing system is intimately related to the balance between the demands of the shoot and the external supply. No doubt, complementary to the cycles of salt content, fluctuations of organic constituents would be revealed upon examination. Apparently these properties can be retained for at least 3 weeks, and at distances of over 6 cm. from the apex. The elucidation of those factors which in the *intact plant* cause this ready removal of electrolytes from root cells which have already accumulated them, and which in *excised roots* would necessitate drastic treatment with perhaps even irreversible changes, represents one of the most difficult and certainly one of the most fundamental of the outstanding problems. In other words, the difficulty which PRIESTLEY and SCOTT faced with respect to the endodermis really applies very strikingly to all the cortical cells. Even if the suggestion they tentatively advanced (based on the effect of an acid reaction in the stele due to carbon dioxide concentration) could be adequately harmonized with present views of the accumulation mechanism or with unpublished data of the effects of carbon dioxide tension upon it, this explanation could hardly apply to the cortex. Having dealt but incompletely with the mechanism by which the root cells accumulate the salts, the problem of their rapid withdrawal and the conditions which determine this can merely be noticed and left unsolved. The evidence, already referred to, that high concentrations in the stele are obtained only if the roots are aerated suggests that this movement also is not unconnected with the metabolism of living tissues.

Summary

1. The absorption of salts by roots is reviewed with special reference to current views upon salt accumulation on the one hand, and physiological anatomy on the other.

[24] During 24 hours' contact with a bromide-free medium, bromide moved to the leaves and the segments near the apex (even as far back as 2 cm.) were almost entirely depleted of the bromide they had previously absorbed (35 milliequivalents per 1000 gm. of fresh weight).

[25] This question has been studied quantitatively in extensive experiments on translocation of bromide in barley plants in the Division of Plant Nutrition, University of California.

2. Quantitative experiments are recorded in which long, unbranched roots of barley (*Hordeum vulgare*), secondary roots of broad bean (*Vicia faba*), and roots of cotton (*Gossypium* sp.) were employed.

3. In roots of barley grown in water culture the potential absorbing surface extends back from the apex to the place where secondary roots appear. The functional absorbing zone is limited by factors which operate upon the cortical cells. The histology of the endodermis and the incidence of vascular differentiation may serve to define the region of most rapid removal, but they are not concerned *directly* with absorption and accumulation of salts by roots.

4. A pronounced longitudinal gradation in the capacity for salt accumulation has been observed. The segments near the apex attain higher concentrations than those more remote. This fact is established for barley plants with both "high-salt" and "low-salt" type of nutrition, secondary roots of bean, and roots of cotton, all of which were grown in water culture.

5. The metabolic and developmental factors involved in the longitudinal gradation of salt accumulation are discussed, and it is concluded that this gradation is an inevitable consequence of the progressive development of cells from the root apex.

6. Interrelations between effects of time, nutrition, and salt accumulation are described. The features common to these and other representative types of absorption/time curves are emphasized. All the time effects are interpreted in terms of the previous nutrition, and the capacity for further growth of the system concerned.

7. Special attention is directed to the probable rôle of the root cortex and to certain outstanding problems which it presents.

These experiments were done in the Division of Plant Nutrition, University of California. Grateful acknowledgment is made to this Division for the facilities provided. The work described relates closely to extensive investigations upon roots then in progress in the Division under the direction of Professor D. R. Hoagland. We have utilized unpublished information and technique derived from these investigations which we now gratefully acknowledge.

The authors' collaboration resulted from a joint stay in the Division of Plant Nutrition during which one of us (Prevot) held a Fellowship under the Commission for Relief in Belgium, Educational Foundation, and the other (Steward) a Rockefeller Foundation Fellowship. The second author accepts the final responsibility for any critical or controversial statements in this presentation.

University of Liége

Birkbeck College, University of London

LITERATURE CITED

1. ANDERSSEN, F. G. Some seasonal changes in the tracheal sap of pear and apricot trees. Plant Physiol. **4**: 459–476. 1929.
2. ARBER, A. The Gramineae. University Press, Cambridge. 1934.
3. AVERY, G. S. Structure and germination of tobacco seed and the developmental anatomy of the seedling plant. Amer. Jour. Bot. **20**: 309–327. 1933.
4. ————————. Structure and development of the tobacco leaf. Amer. Jour. Bot. **20**: 565–592. 1933.
5. BERRY, W. E., and STEWARD, F. C. The absorption and accumulation of solutes by living plant cells. VI. The absorption of potassium bromide from dilute solution by tissue from various plant storage organs. Ann. Bot. **48**: 395–410. 1934.
6. BRYANT, A. E. Comparison of anatomical and histological differences between roots of barley grown in aerated and non-aerated culture solutions. Plant Physiol. **9**: 389–391. 1934.
7. CRIDER, F. J. Selective absorption of ions not confined to young rootlets. Science n.s. **78**: 169. 1933.
8. HENDERSON, L. Relation between root respiration and absorption. Plant Physiol. **9**: 283–300. 1934.
9. HIBBARD, P. L. The chromic acid method for estimation of small amounts of bromine. Ind. & Eng. Chem. **18**: 57–60. 1926.
10. HOAGLAND, D. R., and BROYER, T. C. General nature of the process of salt accumulation by roots with description of experimental methods. Plant Physiol. **11**: 471–507. 1936.
11. ————————, and DAVIS, A. R. The intake and accumulation of electrolytes by plant cells. Protoplasma **6**: 610–626. 1929.
12. JACKSON, VIOLET G. Anatomical structure of the roots of barley. Ann. Bot. **36**: 21–39. 1922.
13. LAINE, T. On the absorption of electrolytes by the cut roots of plants and the chemistry of plant exudation sap. Acta Bot. Fennica **16**: 1–64. 1934.
14. LUND, E. J., and KENYON, W. A. Relation between continuous bioelectric correlation potentials in growing root tips. Jour. Exp. Zool. **48**: 333–357. 1927.
15. PENSTON, NORAH L. Studies of the physiological importance of the mineral elements in plants. III. A study by microchemical methods of the distribution of potassium in the potato plant. Ann. Bot. **45**: 673–692. 1931.
16. POPESCO, ST. Recherches sur la region absorbante de la racine. Bull. Agr. Buc. **4**: 1–136. 1926.
17. PREVOT, P. Localisation de la region d'absorption de la racine. Bull. Soc. Roy. Sci., Liége **6–7**: 243–246. 1935.

18. PRIESTLEY, J. H. Cell growth and cell division in the shoot of flowering plant. New Phytol. **28**: 54–81. 1929.
19. ————, and TUPPER-CAREY, R. M. The water relatio plant growing point. New Phytol. **21**: 210–229. 1922.
20. ROBBINS, W. J. Cultivation of isolated root tips and stem t sterile conditions. Bot. Gaz. **73**: 376–390. 1922.
21. ————. Effect of autolysed yeast and peptone on gro excised corn root tips in the dark. Bot. Gaz. **74**: 59–79. 1922.
22. ROSENFELS, R. The absorption and accumulation of potassium brom by *Elodea* as related to respiration. Protoplasma **23**: 503–519 1935.
23. SABININ, D. A. On the root system as an osmotic apparatus. (Cited from a translation from the Russian.) Perm Univ. Bull. **4**: 1–136. 1925.
24. SCOTT, L. I. The root as an absorbing organ. II. The delimitation of the absorbing zone. New Phytol. **27**: 141–174. 1928.
25. ————, and PRIESTLEY, J. H. The root as an absorbing organ. I. A reconsideration of the entry of water and salts in the absorbing region. New Phytol. **27**: 125–140. 1928.
26. SMITH, G. H. Anatomy of the embryonic leaf. Amer. Jour. Bot. **21**: 194–209. 1934.
27. STEWARD, F. C. Diffusion of certain solutes through membranes of living plant cells and its bearing upon certain problems of solute movement in the plant. Protoplasma **11**: 521–557. 1930.
28. ————. The absorption and accumulation of solutes by living plant cells. V. Observations upon the effects of time, oxygen and salt concentration upon absorption and respiration by storage tissue. Protoplasma **18**: 208–242. 1933.
29. ————. Mineral nutrition of plants. Ann. Rev. Biochem. **4**: 519–544. 1935.
30. ————, BERRY, W. E., and BROYER, T. C. The absorption and accumulation of solutes by living plant cells. VIII. The effect of oxygen upon respiration and salt accumulation. Ann. Bot. **50**: 345–366. 1936.
31. WAGNER, N. Über die Mitosenverteilung im Meristem der Wurzelspitzen. Planta **10**: 1–27. 1930.
32. WEAVER, J. E. Root development of field crops. McGraw-Hill Book Co., New York. 1926.
33. WHITE, P. R. Influence of some environmental conditions on the growth of excised root tips of wheat seedlings in liquid n edia. Plant Physiol. **7**: 613–628. 1932.
34. ————. Potentially unlimited growth of excised tomato root tips in a liquid medium. Plant Physiol. **9**: 585–600. 1934.

RELATION OF TEMPERATURE AND TIME TO CARBON DIOXIDE PRODUCTION AND GROWTH IN CONTINUOUSLY AERATED MALT-AGAR CULTURES OF *POLYSTICTUS VERSICOLOR*[1]

THEODORE C. SCHEFFER[2]

(WITH SEVEN FIGURES)

Introduction

In the present paper are reported results of an experimental study concerning the influence of temperature and length of incubation period on vegetative growth and carbon dioxide production in continuously aerated cultures of a strain of a wood-destroying fungus, *Polystictus versicolor* (Linn.) Fr., growing on malt-agar substrate in darkness. These results are preceded by a somewhat detailed statement concerning several important features of specially devised apparatus and procedure. This study constituted the first part of a more extensive and complicated series of experiments on the oxygen-temperature relations of this same strain of *P. versicolor* growing under the general conditions here described, in which the partial pressure of oxygen was a third experimental variable. A study of this particular fungus with reference to its oxygen-temperature-time relations was undertaken with the aim of contributing to our present knowledge of the fundamental physiology of such organisms, and also in preparation for a broader but much less intensive experimental comparison of a number of different wood-attacking fungi in respect to their oxygen requirements. Reports on these two additional studies are in preparation.

Vegetative growth and carbon dioxide elimination are among the most significant of the easily measured features of the performance of a fungus, and additional information concerning these two very general processes and their interrelations should contribute fundamentally to the advancement of general physiology and ecology, as well as to that of mycology and phytopathology and their related fields of application. No attempt was made to study any of the many other dynamic criteria by which various degrees and kinds of physiological activity in an organism might be compared.

[1] Botanical contribution from the Johns Hopkins University, no. 134.

[2] The research for this paper, together with related experiments dealing with wood-inhabiting fungi, which are to be reported in other papers, was done between October, 1934, and September, 1935. The work was made possible by the grant of a fellowship in the biological sciences from the National Research Council, and by the cooperation of the Johns Hopkins University, where facilities of the Laboratory of Plant Physiology were made freely available.

The influences that constituted the experimental background complex of the environment, which were essentially constant throughout this study, were: a specific nutrient and water supply in the substrate; the partial pressures of oxygen and aqueous vapor that characterize ordinary air when saturated with water, and the practical absence of carbon dioxide in the aerial surroundings; absence of light and of injurious substances and competing organisms; and a prescribed preliminary treatment of the fungus preceding each experiment. The use of a continuous flow of gas through culture chambers is somewhat new for this kind of study. It insures the maintenance of any specified aerial environment throughout an experiment and permits the collection and measurement of the CO_2 produced.

The experimental variables were temperature and length of period of growth. The possible influence of changes or fluctuations in temperature was not studied. In each culture unit temperature was maintained at a practically constant value throughout the entire experiment period, which was 5 days in length, and five different temperatures were employed, ranging above and below the optimum for vegetative growth. The time factor requires special attention, not only in connection with computations of growth rates and rates of carbon dioxide production but also with reference to the physiological aging of the mycelial mats.

While publications dealing with the influence of temperature on growth and respiration are not infrequent in the literature of plant physiology, one finds but few papers that might, in themselves, contribute toward generalizations. Considerations of concurrent growth rates and rates of carbon dioxide evolution seem especially to have been limited. Several papers bearing on temperature influence are to receive mention in the following sections, but it may be noted here that the reports of BONNIER and MANGIN (**2**), BOER (**1**), and WASSINK (**16**) are the only ones encountered in which is described the use of continuously flowing gases for fungus cultures. The objectives of the writers just mentioned were, however, quite different from those envisaged in this study. The technique of these experiments resembled in many respects that employed by MACK (**13**), and MACK and LIVINGSTON (**14**) in connection with their studies on temperature-oxygen relations of growth and carbon dioxide production in very young wheat seedlings. Their papers were very useful in the planning of these and subsequent experiments with flowing gas.

Polystictus versicolor was selected in the present instance because it is one of the most important among fungi that cause decay in dead hardwood material throughout the United States, and because it is propagated readily and grows rapidly on agar substrate under experimental conditions that may be easily provided, producing an abundance of aerial hyphae that would be freely exposed to a gas flowing through the culture tube. Further-

more, this organism is specially suitable for study of this sort because it may be grown for comparatively long periods of time as an enlarging mat of mycelium, without the appearance of special reproductive phases. The particular strain of *P. versicolor* used was obtained as a transfer from a stock culture at the United States Bureau of Plant Industry. It had been originally isolated from maple sapwood.

Experimentation

General considerations

Two similar experiments with ordinary air were conducted, each lasting 5 days, and observations on growth and CO_2 production were made at 24-hour intervals. Fairly precise artificial control of the two experimental variables (temperature and time) was provided, while all environmental influences excepting temperature were essentially constant throughout all cultures and both experiments. These constant background conditions have already been mentioned. The influence of light was avoided by carrying out the experiments in darkness, except that each culture was temporarily illuminated for a very few minutes each day, to permit the making of growth records. Light has been shown (**2**) to influence CO_2 production in fungi and, although no record of a specific light effect on the rate of vegetative growth in these organisms has been found, some influence of this factor is at least a possibility. The humidity of the aerial environment may also influence respiration (**2**), and possibly growth rate also, in fungi that are partly exposed to air, and, of course, the maintenance of the water content in the substrate and of the associated concentrations of the solutes present is mainly dependent on the water-vapor pressure of the air in the culture chamber. Any variable influence on this account was virtually prevented, since the air stream continuously passing through each culture tube was always saturated with water vapor. That suitable uniform and reproducible nutrient conditions are essential in such a study as this needs no special comment. Many investigators have remarked that CO_2 pressure may influence growth in fungi, especially when comparatively high. Since rates of CO_2 production were measured by means of continuous removal and absorption of that gas, its partial pressure in the culture tubes was always maintained at a very low value. That the experimental cultures must be pure (without competing organisms) and that effectively injurious concentrations of potentially toxic substances were to be avoided, is self evident; as is also the requirement that preliminary treatment should be alike for all cultures. Although these background conditions were not analyzed and quantitatively specified, the standard procedures of the experimentation were such that the background complex was satisfactorily reproduced for

the second experiment reported here and for many other experiments with flowing gas. It should be essentially reproducible at any future time if the same procedures are adequately adhered to.

Culture technique

Fungus employed.—Subsequent to its isolation from wood, the strain of *P. versicolor* used in this study had been perpetuated in stock cultures, in test tubes or Petri dishes, on malt-agar gel similar to the experimental substrate used in these experiments. The temperature optimum for vegetative growth of this species appears to lie between 28° and 30° C., while the maximum is about 35° C. These cardinal temperature values may be somewhat different for different strains and perhaps for the same strain under different sets of nutritional conditions; the species appears to show a comparatively broad optimal temperature range and a short supra-optimal range below the temperature maximum (**8**).

The experimental stock cultures, which were in Petri dishes, were renewed by transfer from time to time throughout the period of these studies (October, 1934 to May, 1935). They were kept in a refrigerator at about 9° C., excepting that new transplants were first kept in darkness at 20° to 25° C. for about 5 days, to permit the mycelial mats to attain adequate size for use as source of inoculum. Pieces of inoculum for the experimental cultures were cut from the outer regions of circular stock-culture mats so as to represent growth of the last three days—*i.e.,* they were taken from within about 3 cm. of the mat margin. Under the conditions of these studies this organism regularly produced a moderately dense white mycelium covering the exposed surface of the agar substrate uniformly to the extreme margin of the area occupied, with an even and well-defined frontal edge that advanced very regularly.

Experimental substrate.—It was realized that a nutrient medium for such studies as this should ideally be so constituted that all of the physiologically influential components might be quantitatively specified or prescribed, to the end that all could be adequately taken into account when different substrates are to be compared and when the standard substrate of one experimental series is to be duplicated in another series, whether by the same experimenter or by another; but such an ideal is of course only very roughly approachable as yet. Fair degrees of development of this strain of *P. versicolor* were obtained in some preliminary trials with a medium prepared from chemically pure inorganic salts, glycerine, and dextrose, together with the bacto-agar mentioned below—but use of the malt extract employed (which is of course an unknown mixture of organic and inorganic compounds) resulted in much more vigorous growth. It was clear from the start that the development of a truly synthetic medium for the artificially

controlled culture of a wood-attacking fungus must constitute a very difficult and time consuming undertaking (**3**), which would be in itself a major research; consequently but little time was devoted to these preliminary trials. Nor was any attempt made to develop a more definite gel than that obtained from the agar used, which also must be considered as an unknown mixture of many substances.

The medium used for the regular experiments, which was uniformly clear and proved to be very satisfactory, was made up with distilled water (from a Barnstead still) so as to contain 24.88 gm. of heat-treated Trommer extract of malt, 0.12 gm. of NH_4NO_3, and 20 gm. of Difco standardized bacto-agar. Since the malt extract, as it came to hand, apparently contained a considerable amount of material that coagulated during the sterilization process and became unevenly distributed, the stock of extract was preliminarily brought to a temperature of 100° C., after which the coagulated material was allowed to settle and only the clear supernatant liquid was used. A stock mixture of heat-treated malt extract and NH_4NO_3, in the requisite proportions, was first prepared sufficient for all three studies mentioned in the introduction. This was kept in a refrigerator, to be drawn upon whenever a new batch of medium was to be made up. During the course of six months there was no evidence that this stock had altered in any way that might affect either growth or CO_2 production.

For each experiment a lot of medium was freshly prepared as follows: Twenty grams of dry agar was first "dissolved" in about 950 ml. of distilled water, by heating in the autoclave for 15 minutes at a pressure of 5 lb. (*i.e.*, at a temperature of about 108° C.) Into the resulting agar sol was then stirred 25 gm. of the stock mixture of malt extract and NH_4NO_3, after which enough distilled water was added to produce a liter of medium. The freshly prepared medium was immediately siphoned into the culture tubes, each of which received about 16 ml., care being taken to have the liquid stand at the same height in all tubes. The tubes were next plugged with cotton in the usual way and autoclaved in upright position for 15 minutes at a pressure of 13 lb. (*i.e.*, at 119° C.), following which they were allowed to cool for 48 hours at room temperature and in horizontal position, to allow the gel to form and become adequately attached to the tube wall, producing a solid agar strip along one side of each tube. The tubes were then ready for inoculation.

CULTURE TUBES AND CULTURE UNITS.—Culture tubes of a new form were devised (**15**) for these studies. The new tube consists of an ordinary test tube 2.5 cm. in diameter and 20 cm. long, modified by a rounded indentation or invagination of the wall on one side near the mouth, as shown in figure 1. A narrow strip of the cylindrical wall, on the same side as the indentation, is ground externally, to facilitate marking with a pencil for growth

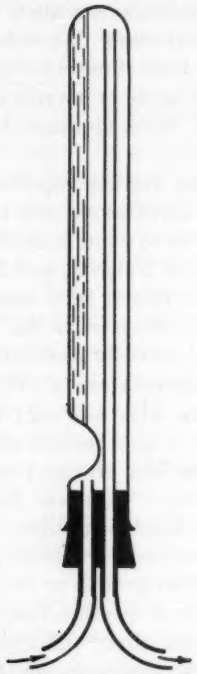

FIG. 1. Vertical section through culture tube designed for continuous gas flow.

records. In the temporary horizontal position of the tubes, while the medium was cooling and solidifying, the indentation formed a dam that prevented escape of medium while the latter was still liquid. The agar strip adhered to the tube wall longitudinally, on the same side as the indentation, extending therefrom to the closed end of the tube, as indicated by shading in figure 1. It was about 15 cm. long and its greatest thickness, along the middle longitudinal line, was about 10 mm., its free surface being about 21 mm. wide throughout its length.

The inoculum was placed on the surface near the middle of the strip and the ensuing mycelial mat expanded longitudinally along the surface in both directions, lateral growth beyond the edges of the strip on to the glass wall being relatively slight. The positions of the two advancing margins, observed through the agar strip, were marked daily on the ground portion of the tube. Throughout the experiment period the culture tubes were vertical, with rounded ends uppermost, and the mycelial mat could extend about 7.5 cm. in each direction, upward and downward, before reaching the ends of the substrate. Thus the room for longitudinal expansion along the

agar surface was considerably greater than would have been possible for radial enlargement of circular mats in any but very large Petri dishes inoculated at the center. Petri dishes are obviously unsuitable for cultures in which a continuous flow of gas is to be provided, as in these studies.

To provide for continuous flow of gas through the culture tubes, the cotton plug that had been introduced immediately after inoculation was replaced by a tightly fitting 2-hole rubber stopper bearing two glass tubes for inlet and outlet (fig. 1), the former terminating close to the stopper while the latter extended nearly to the closed end of the culture tube, without approaching the agar strip. Ten culture tubes that were to receive like treatment simultaneously were placed vertically side by side, along with four unmodified test-tubes, like those from which the culture tubes had been made. These were likewise equipped for entrance and exit of the gas stream and all fourteen were connected in series by short rubber-tubing couplings and were supported in a rack, as shown in figure 2. Each assembly of fourteen tubes constituted a culture unit, of which there were regularly five in each experiment, one for each of the five different maintained temperatures employed.

For each culture unit incoming air was freed from CO_2 and H_2O vapor by being serially led through two of the unmodified test-tubes just men-

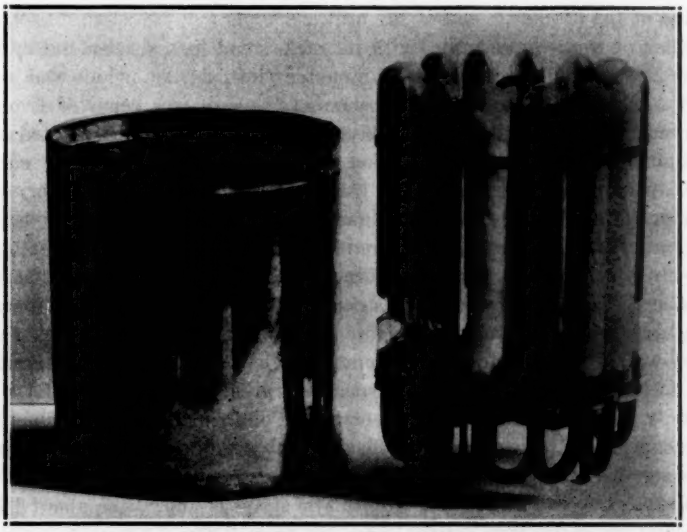

FIG. 2. Culture unit of ten culture tubes and four additional tubes for conditioning the entering gas; also supporting rack and water bath.

tioned, which were charged with soda-lime. Near the outlet of the second tube were a few cubic centimeters of ascarite to serve as telltale, showing (by change from light to dark color as this material approached CO_2 saturation) when renewal of the soda-lime charges was necessary. The dry, CO_2-free air next bubbled through a phenolphthalein-colored aqueous solution of very dilute $Ba(OH)_2$ in the third unmodified test-tube, where the upward passage of bubbles was retarded by glass beads. Retention of the pink phenolphthalein color throughout the 5 days of each experiment served to show that no CO_2 had passed the soda-lime tubes. As the gas bubbled through the third tube it also took up water vapor and was brought to approximate water saturation for the prevailing temperature; *i.e.*, to equilibrium with the prevailing water-vapor pressure in the culture tubes. Consequently the continuous passage of the gas stream along the agar surfaces produced no significant drying or wetting of the latter. The fourth of the unmodified test-tubes was filled with non-absorbent cotton, which acted to filter out any spores or other biological contaminants that might pass the first three tubes. This filter tube, with its cotton filling, was autoclaved prior to its introduction in the culture unit. From the filter tube the gas passed consecutively through the 10 culture tubes, being then conducted directly to a CO_2 absorber, where CO_2 from all ten cultures was collected for titration at daily intervals. It was found that no cotton filter was needed at the discharge end of the unit.

Each of the culture units, with its rack, stood in a sheet-metal cylinder about 22 cm. high and 19 cm. in diameter (left, fig. 2), which was nearly filled with water. During the experiment the unit was removed from this bath only for a brief period each day, when it was temporarily lifted out to facilitate the regular observations on growth. This water seal proved to be effective in preventing significant exchange of gases between exterior and interior of the culture units when gases other than ordinary air were used. The water bath also served to minimize for the cultures any slight temperature fluctuations that may have occurred in the air of any temperature chamber. The portions of the gas conduit that were within a temperature chamber, leading the air stream into and out of the culture unit, were of heavy-walled rubber tubing, just long enough (about 35 cm.) to permit the unit to be lifted out of its water bath and raised above the top of the temporarily opened chamber when observations on growth were to be made; all but about 12 cm. of each of these flexible tubes was regularly submerged in the water bath.

PREPARATION OF CULTURES.—Prismatic strips of inoculum, about 16 mm. long and about 1.5 mm. square, were cut from 3-day-old mycelial mat of a stock Petri-dish culture, so that each strip included the underlying agar. One of these was placed crosswise near the middle of the substrate strip in

each culture tube, the mycelial surface being uppermost when the tube was horizontal with its agar strip below. Thus the advancing margins of new growth, at right angles to the long axis of the culture tube, were nearly straight from the start and any linear increment of advance was consequently very nearly proportional to the corresponding areal increment. These strips of inoculum were found to be more satisfactory than the nearly cubical or irregularly shaped bits commonly employed, since the latter characteristically produce a curved margin of growth.

Following inoculation, the cotton-plugged culture tubes were kept horizontal in darkness for approximately 15 hours, at a temperature of about 26° C., to allow the new growth to become established on the fresh agar. The 14 tubes of each culture unit were then assembled in the rack, being held in place by means of a large rubber band, and the cotton plugs were replaced by the rubber stoppers and glass-tubing attachments mentioned above. These operations were always performed with the tube mouths directed downward, to protect the interiors from mold spores and bacteria that might otherwise settle in from the outside air. Then all 14 tubes of each unit were joined by the short rubber-tubing couplings already mentioned, and the unit was placed in its water bath, the two openings for gas being temporarily sealed against entrance of water, by means of rubber caps that were eventually replaced by the heavy-walled rubber tubes mentioned above.

The rubber stoppers and their glass tubes were always autoclaved before being introduced, but autoclaving was found to weaken the rubber-tubing used; therefore the couplings were sterilized by dipping in a 0.25-per cent. aqueous solution of $HgCl_2$. No opportunity was offered for this antiseptic to reach the substrate in the culture tubes, since the stoppered ends of the latter were always directed downward.

After the units, in their respective baths, had been introduced into the temperature chambers and after the air streams had been started through the units, an adjustment period of about 15 hours was allowed to elapse before the beginning of the first observation period, after which observations of growth and CO_2 production were made at 24-hour intervals for 5 days.

TEMPERATURE CONTROL.—The five different temperatures used (17.5°, 21.5°, 25.5°, 29.5°, and 33.5° C., with maximal fluctuations not exceeding plus or minus 0.5° C.), were provided by means of the original LIVINGSTON-FAWCETT (**11**) electrically controlled, 7-chamber temperature apparatus, which has been in satisfactory use in this laboratory every year since its installation in 1919. This consists essentially of seven vertical sheet-metal cylinders, about 44 cm. high and 28 cm. in diameter, each of which is surrounded below and laterally by water that is continuously stirred. These

stand side by side in a row, adjacent water jackets being separated by sheet-metal septa through which heat is conducted from one water jacket to the next. Electrically controlled heat is supplied at one end of the row and heat is removed at the other end by means of a small automatically regulated refrigeration machine, the whole series of chambers being inclosed in a chest-like case with insulated walls. Each cylinder or chamber is provided with a removable insulated lid that gives access to its interior from the top. (A portion of the case and the 7 chamber lids are visible in figure 3.) The 2 thermostats that control heating and cooling respectively are

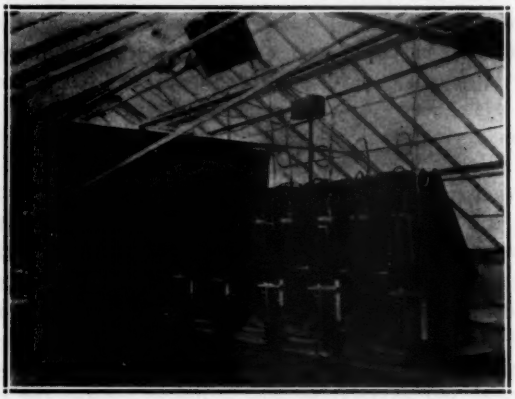

FIG. 3. General view of temperature chambers, CO_2 absorbers and regulators for gas flow.

adjusted to maintain a requisite temperature in each of 2 accessory water tanks, one at each end of the row, and the intervening chambers provide a graduated series of temperatures, the warmest chamber being adjacent to the heating tank while the coolest is adjacent to the cooling tank. Only 5 of the chambers were used in these studies. Each was provided with 2 tubes of block tin, which served as inlet and outlet for the continuous air stream that was conducted to and from the single culture unit in each chamber. Somewhat above and at the side of each temperature chamber was the CO_2 absorber for the culture unit in that chamber. (The absorbers and the 5 large bottles of the air-flow regulators may also be seen in figure 3.) Titrations of the CO_2 absorbed were accomplished without opening the chambers.

REGULATION OF THE AIR FLOW.—Although all five of the culture units of an experiment were operated simultaneously, each had its own separate air stream. Air entered each unit from the greenhouse room in which these arrangements stood, being then led to the corresponding CO_2 absorber,

from which it passed into an aspirator bottle provided with a specially devised regulator that maintained a rate of flow of about 15 liters a day. Spent air accumulated in the aspirator bottle during each day's run, being discharged when the bottle was refilled with water for the succeeding day. Differences among the five systems with respect to their resistance to gas flow were satisfactorily compensated by separate adjustment of the respective flow regulators. The 15-liter daily rate of flow was regularly maintained for all five culture units and throughout both experiments, with fluctuations of not more than plus or minus 6 per cent.

The flow regulator used consists of an aspirator bottle of Mariotte type (*A*, fig. 4) provided with accessories to maintain the requisite suction (*B*, fig. 4). The mean rate of gas entrance into the bottle through tube *a* and

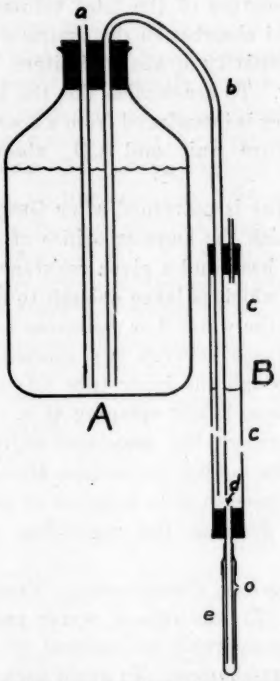

FIG. 4. Vertical section through gas-flow regulator.

the corresponding rate of water escape through opening *o* are alike; consequently control of the rate of water escape results in control of the rate of gas flow through the associated culture unit and CO_2 absorber. Siphon *b* discharges water into the auxiliary tube *c* (about 50 cm. long, with bore

about 2.5 cm.), which is open above to the outside atmosphere. Thence water passes into the drip regulator (*e*), and it escapes through orifice *o*. For a given setting of the drip regulator the effective suction is determined by the position of the bottle with reference to the water level in the auxiliary tube. With the relative positions of these fixed, any change that may occur in air pressure within the bottle, in water-flow resistance of the drip regulator or in gas-flow resistance of the culture-absorber system, produces a corresponding change of water level in *B*, thus tending to overcome the effect of the change. Consequently the requisite mean rate of air flow is satisfactorily maintained when the drip regulator has been properly adjusted.

The descent of the free water surface in the bottle during any interval naturally furnishes a measure of the total volume of air that has moved through culture unit and absorber to the aspirator in that interval. Since the bottles used had a capacity of about 19 liters, it was necessary to refill them but once each day. To accomplish this the bottle stopper is raised a short distance while water is introduced from a hose, the air current through the corresponding culture unit and CO_2 absorber being temporarily interrupted.

The drip regulator (*e*) is patterned after GREGORY'S arrangement (**7**), on the principles of which the annular orifice of ZINZADZE (**17**) is based. For a given hydrostatic head and a given resistance to air flow, the rate of drip at the opening *o* (which is large enough to offer no significant resistance) is determined by the water-flow resistance interposed by the walls of the annular capillary space between two concentric glass tubes. Water enters the regulator through the inner tube (*d*) and then passes upward through the annular space, finally escaping at *o*. To increase or decrease the rate of gas flow through the associated culture-absorber system the outer glass tube, with its rubber connection above, is slipped upward or downward on the inner tube, so as to lengthen or shorten the annular space and thus increase or decrease the water-flow resistance of the drip regulator.

As has been emphasized by CHRISTIANSEN, VEIHMEYER, and GIVAN (**4**), and also by ZINZADZE (17), the rate of water passage through a narrow orifice may fluctuate considerably on account of changes in viscosity of water, due to temperature changes. To avoid such temperature effects in a device of the type here described it would be necessary to introduce some arrangement by which the water temperature within the annular space might always be the same, or to readjust the orifice from time to time according to temperature changes. However, no special precautions of that sort were taken in the present experimentation.

As to the suitability of the 15-liter daily rate of air flow which was regularly employed, that rate may be considered first with reference to removal of CO_2 from the cultures and then with reference to O_2 supply. Preliminary tests with well-developed and vigorous cultures of this strain of *P. versicolor* at 20° C. produced CO_2 as rapidly with an air flow of 4 liters a day as with a flow twice as rapid. This indicates that the 4-liter rate of flow was adequate to remove CO_2 as rapidly as it was produced in these cultures, and it is safe to suppose that the 15-liter rate regularly employed was surely adequate, even under the most favorable conditions for metabolic activity and with the most extensive mycelial mats developed in the 5-day experiment period. The maximal daily output of CO_2 from any culture unit, even with nearly pure O_2 (used in later experiments), was only 75 ml., which is but 0.5 per cent. of the volume of air regularly passed through a unit each day. Furthermore each charged and stoppered culture tube had a gas capacity of only about 65 ml. and consequently the 15-liter rate of flow corresponded very nearly to an hourly renewal of the air in the 10 culture tubes of each culture unit. Of course, the air entering any tube beyond the first in each culture unit carried the CO_2 that had just been swept from preceding tubes of that unit; only the first tube in a unit received air quite free from CO_2. In view of the relatively very rapid rate at which air moved through each unit, this consideration may safely be neglected, however, and the partial pressure of CO_2 at the surfaces of the mycelial mats must have been exceedingly low in all cultures, and sensibly constant.

Because the rate of air flow and the proportion of O_2 in the air stream were both practically constant throughout the tests here considered and because gas dilution due to CO_2 production was negligible, it follows that the O_2 supply and the attendant partial pressure of O_2 in the culture tubes were both practically constant. With a total air pressure in the culture tubes of 755 mm. of mercury column and a mean volumetric O_2 content of 20.8 per cent. (see below), the 15-liter rate of air flow brought to each culture unit about 3 liters of O_2 per day. That is surely very greatly in excess of the maximal daily rate of O_2 absorption in a unit, which may be estimated as of the same order of magnitude as the maximal daily rate of CO_2 production, 75 ml. It is therefore safe to say that O_2 entered the culture units more than thirty-five times as rapidly as it was absorbed by the most vigorously absorbing mats. Also, in the preliminary tests just mentioned the 8-liter rate of air flow gave vegetative growth no more rapid than was given by the 4-liter rate. It therefore seems clear that the 15-liter rate was much more than adequately rapid in all these tests, not only for removing the CO_2 produced but also for supplying O_2 to the mycelial mats.

PARTIAL PRESSURES OF O_2 IN AIR STREAMS AT DIFFERENT TEMPERATURES.—The air stream passing through the culture tubes was always practically

saturated with water vapor, but of course the partial pressure of H_2O varied somewhat with temperature, being equivalent to the corresponding vapor tension of liquid water. For the five temperatures employed, the vapor-tension values, as interpolated in table LXXIX of Van Nostrand's Chemical Annual (1909) and expressed as millimeters of a mercury column, are approximately as follows: 17.5° C., 14.9 mm.; 21.5° C., 19.1 mm.; 25.5° C., 24.3 mm.; 29.5° C., 30.7 mm.; and 33.5° C., 38.5 mm. It is clear that, for any given barometric pressure, the partial pressures of O_2 and N_2 in the air stream must have varied slightly according to the temperature used, being somewhat greater with higher than with lower temperature.

Since the gas stream was produced by suction in these experiments with air, the air pressure in the culture tubes was always somewhat less than the prevailing barometric pressure on the outside; it was about 755 mm. (Barometric fluctuations accompanying weather changes were not taken into account, since these are surely negligible in such experimentation as this.) The outside air, from which the flowing gas for the two experiments here considered was drawn, was analytically found to have a volumetric O_2 content of 20.9 per cent. in one case and of 20.6 per cent. in the other; for both experiments this is taken as 20.8 per cent.

The approximate partial pressure of O_2 in the air stream entering the first culture tube of a unit at 17.5° C. is computed by subtracting 14.9 mm. (H_2O) from 755 mm. (total air pressure) and taking 20.8 per cent. of the remainder, the result being 153.9 mm. The similarly computed partial pressures of O_2 for each of the five experimental temperatures are as follows: 17.5° C., 153.9 mm.; 21.5° C., 153.1 mm.; 25.5° C., 152.0 mm.; 29.5° C., 150.7 mm.; and 33.5° C., 149.0 mm. Since these differences are so small, the partial pressure of O_2 may safely be considered as 152 mm. for all five temperatures, with a plus or minus variation of less than 2 per cent.,—which is surely within the limits of experimental error in this kind of experimentation.

TUBING AND CONNECTIONS.—Reverting to the description of the experimental apparatus, it should be mentioned that the whole of the gas conduit (from bottle intake to culture-unit intake) was of glass, copper or block tin, excepting where rubber tubing was required for couplings or to permit necessary flexibility. The rubber tubing used was of "pure gum," with bore about 4 mm. and external diameter about 10 mm. The metal tubing used has an external diameter about 6 mm. and a bore about 3.5 mm. It was initially tested for leaks, with a differential air pressure of 700 mm. of mercury column. As has been noted, all rubber connections within each culture unit were protected by means of a water-bath seal. All other rubber connections were coated exteriorly with spar varnish (Valspar) and were

ligatured with wire unless protected by means of the glycerine seals described below.

MEASUREMENT OF CO_2 PRODUCTION

CO_2 ABSORBERS.—The type of CO_2 absorber employed, which is a modification of Pettenkofer's apparatus, follows in the main a design now in use in the chemical laboratories of the Johns Hopkins University, but the glycerine seal and the burette collar used with it were specially developed for these experiments. The essentials of the absorbers used are shown in figure 5. The flask was generally charged with 25 ml. (30 ml. or 35 ml. when

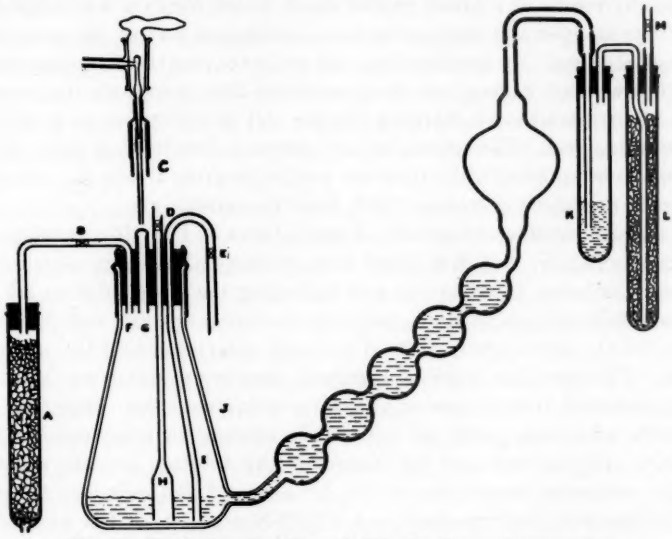

FIG. 5. Vertical section through CO_2 absorber and accessories.

unusually rapid CO_2 production was expected) of standard absorber solution, to which was added enough distilled water to make the solution nearly fill the oblique side arm when gas was flowing. Gas from a culture unit enters through the inlet (*D–H*) and bubbles continuously into the free space of the flask, whence it passes intermittently out through the side arm. (The lower end of the inlet tube is enlarged at *H* so as to have an internal diameter of about 12 mm., to avoid the possibility of serious plugging with precipitated $BaCO_3$.) From the lateral arm of the absorber the gas bubbles through the telltale tube *K* (which is partially filled with very dilute $Ba(OH)_2$ solution (about 2 gm. per liter) colored with phenolphthalein), where maintenance of the color gives assurance that CO_2 has not passed the

absorber. Soda-lime tube *L* protects the absorber from possible entrance of CO_2 from the apparatus beyond clamp *M*, which is open when gas is flowing. An additional soda-lime tube (*A*) is temporarily brought into connection (by opening clamp *B*) during the operations of charging and emptying the absorber and while titration is in progress.

Absorber solution is discharged through tube *I*, clamp *E* being temporarily opened for that operation, and the absorber is charged through tube *G*, through which also standard acid solution is added in titration, this tube being ordinarily closed with a rubber cap, as shown in the figure. A glycerine seal covers the top of the flask stopper, the glycerine being held in place by means of a broad rubber band, which forms a wall around the top of the stopper and also serves as an additional seal at the junction of flask and stopper. During titration and while the absorber is being charged tube *G* is without its cap and the glass collar that surrounds the extended outlet of the titration or charging burette (*C*) is lowered so as to dip into the glycerine seal. This glycerine seal between burette and flask allowed the flask to be agitated while titration was in progress and at the same time effectively prevented entrance of CO_2 from the outside air.

STANDARD ABSORBER SOLUTION.—Fifteen liters of $Ba(OH)_2$ solution (approximately 0.2 N) was first freed from whatever $BaCO_3$ it contained by allowing the latter to settle out and siphoning the clear solution off. To this was added 4.5 gm. of $BaCl_2$ per liter, to reduce solution and hydrolysis of the $BaCO_3$ subsequently formed through absorption of CO_2 in the absorbers. The resulting stock of standard absorber solution was kept in a bottle, protected from access of CO_2 by a soda-lime tube. Repeated tests showed its alkalinity, which did not alter in storage for many months, to be 0.2285 N. The burette used for measuring this solution into the absorbers was also protected from access of CO_2 by means of two soda-lime tubes.

ACID SOLUTION FOR TITRATION.—A 0.2285-N solution of HCl was used in titrating for CO_2 absorption in the absorbers. A 15-liter stock of this solution was prepared at the beginning, and this was stored in a rubber-stoppered bottle. Repeated standardization, by means of ovendry Na_2CO_3 and methyl orange, showed that the acidity of this stock did not change in storage.

TECHNIQUE OF CO_2 MEASUREMENTS.—For each of the five culture units in each experiment, the CO_2 accumulated as $BaCO_3$ in the corresponding absorber was measured daily by titration in the usual way, with phenolphthalein as indicator. Referring to figure 5, the titration procedure was as follows: Clamps *D* and *M* were closed and the absorber was temporarily tilted so as to cause the liquid in the side arm to return to the flask. Then clamp *B* was opened, the cap from the upper end of *G* was removed, and 3 drops of alcoholic solution of phenolphthalein (1 gm. in 100 ml.) was intro-

duced into the flask through *G*. after which the 0.2285-N HCl was introduced drop by drop through *G* from the acid burette (fig. 3, at left), while the absorber solution was vigorously agitated. When the indicator color faded, addition of acid solution was suspended, the cap was replaced on *G*, clamp *M* was opened and the absorber was tilted forward and back so as to rinse its side arm and return the rinsings to the flask. Then clamp *M* was again closed, the cap was removed from *G* and addition of acid solution was resumed and continued till the indicator end point was reached, when the total volume of acid solution used was recorded for use in computing CO_2 production for the preceding day. Only one rinsing of the side arm was required.

After a titration had been completed, clamp *E* was opened (*G* being closed) and the neutralized liquid was siphoned out through tube *I*, slight mouth suction being usually required to start the flow. No attempt was made to remove the $BaCO_3$ left adhering to the absorber walls, since that has been found (**13**) to have no significant effect on titration results of this kind.

In recharging an absorber, fresh absorber solution and the requisite amount of distilled water were introduced through *G*, clamp *E* being closed. As soon as a flask had been recharged clamp *B* and the opening of tube *G* were closed and clamps *D* and *M* were once more opened, permitting resumption of air flow, which continued during the succeeding observation period, until the next titration was to be made.

At the end of each observation interval air flow was discontinued simultaneously through all five culture units and their respective CO_2 absorbers, and the five absorbers were cared for successively and in regular order. As soon as the fifth had been recharged air flow was simultaneously resumed in all five cases, being continued throughout the succeeding observation period. It required about 45 minutes to care for the five absorbers, during which time the cultures were without air flow, but this interruption occurred only once a day and when flow was resumed the stagnant gas in conduits and culture tubes was soon delivered to the absorbers; thus CO_2 produced in the short period without air flow was eventually measured as pertaining to the succeeding period of flow.

In spite of the exercise of a great deal of care to prevent any gas interchange between the air stream and the atmosphere outside of the apparatus, blank tests (regular in all respects excepting that the culture tubes were without either agar strip or mycelium) showed what might be interpreted as the result of a slight but consistent leakage of CO_2 into the apparatus from the outside. Regular titrations of these blanks tests indicated that CO_2 had somehow reached the absorber at consistent rate of about 0.75 mg. per day. The question as to whether these results might perhaps be explained

otherwise than by reference to leakage, or whether leakage may actually have occurred (as perhaps through the walls of the rubber tubing used—for CO_2 is soluble in rubber gum as well as in water) was not studied, however. Because the apparent systematic error thus introduced is relatively so small and appears to have been so constant, it has been approximately cared for by the introduction of a uniform correction; from the amount of CO_2 produced each day, as computed from the titration records, was subtracted 0.75 mg., to allow for that amount of CO_2 as derived from some unknown source.

From each titration record was computed the weight of CO_2 (in milligrams) that had been received from the corresponding culture unit (ten culture tubes) in the preceding 24-hour interval. There were thus five CO_2 values, representing respectively the five successive days of the experiment period, for each of the five culture units of each experiment; i.e., for each of the five maintained temperatures that were tested. After each of these daily outputs of CO_2 had been decreased by 0.75 mg., as has just been mentioned, the two corresponding values (one from each of the two experiments with air) were averaged in each case. The resulting averages constitute a single series of twenty-five numerical indices of daily CO_2 production, representing both experiments together, which are inscribed on the dotted-line graphs of figure 6, which will receive attention below.

Growth observations and measurements

Observations on the growth of the mycelial mats were made, without interrupting the flow of air, for 24-hour periods that essentially coincided with the corresponding intervals of CO_2 measurement. In recording growth, the temperature chambers were opened, one at a time and in regular order, and each culture unit was lifted and held above its chamber while the positions of the upper and lower margins of each fungus mat were quickly marked (with a lead pencil) on the ground portion of the culture tube. The unit was then promptly returned to its water bath and the temperature chamber was closed. An electric lamp was always used in this operation and each unit was out of its chamber only about 4 minutes each day.

At the termination of each experiment, when all culture units were finally removed from their respective temperature chambers and water baths, the growth increments and mat lengths indicated by the pencil marks were measured with a millimeter scale and recorded. For each 24-hour period there were two concurrent daily growth increments (upward and downward) for each culture and, since each unit embraced ten cultures, there were twenty increments for each unit. Because it was found that there was no consistent difference between upward and downward rates of mycelial advance along the substrate strips, these twenty values were averaged in each case, and

consequently each experiment gave twenty-five average growth increments, one for each temperature for each day. There were two sets of these values, a set for each of the two experiments with flowing air, and the two corresponding values indices (one from each experiment) were finally averaged in each case, the result being a single series of twenty-five average growth rates, one for each temperature for each day. The average mat length at the end of each day was also computed for each culture unit, and these average lengths are inscribed on the continuous-line graphs of figure 6.

Results

Growth upward and downward

There was no evidence of gravitational influence on the rate of mycelial advance, whether in the two experiments with flowing air, with which this paper deals, or in the more extensive series of experiments in which essentially pure N_2, essentially pure O_2, and various artificial mixtures of these gases were employed for the continuous gas flow. In all these experiments each mycelial mat always advanced upward and downward at approximately the same rate. The rate of growth showed no relation to the position of the culture tube, whether horizontal or vertical, and it appeared also that the hyphae exhibited no geotropism. In this connection it may be noted that the fruiting bodies of this fungus show pronounced dorsiventrality.

The technique of these experiments did not furnish means by which rates of CO_2 production for upwardly growing and downwardly growing mats might be separately measured and compared; for at any time the upper half of each mat had been produced by upward growth and the lower half had resulted from downward enlargement. Since there was no gravitational influence on growth, it seems highly improbable that gravitation exerted any influence on CO_2 production.

Growth and CO_2 production in relation to age and extent of mycelial mats

Time graphs of mat length and daily CO_2 production are shown in figure 6, where each pair of graphs represents a single temperature. Each pair has its own base line, as indicated at the left. Ordinate values are inscribed on the graphs; in plotting, 1 mm. and 2 mg. are represented by the same ordinate distance. The continuous-line graphs, which represent mat length, show clearly that mycelial advance along the agar strip was essentially proportional to time at every tested temperature for these graphs are all essentially straight lines. The mean rate of upward and downward enlargement of any mycelial mat remained essentially the same throughout the 5-day experimental period, being unrelated to the age or extent of the mat. This observation is like that recorded by FAWCETT (6) for his long-tube cul-

tures of parasitic fungi. The advancing hyphae were always extending on to and into fresh nutrient medium, which was apparently still uncontaminated by any influential substances that may have arisen from the more mature portions of the mat. These statements apply for every one of the five tested temperatures in these two experiments with flowing air, and also for all experiments with other gases (excepting when the partial pressure of oxygen was too low to permit measurable growth).

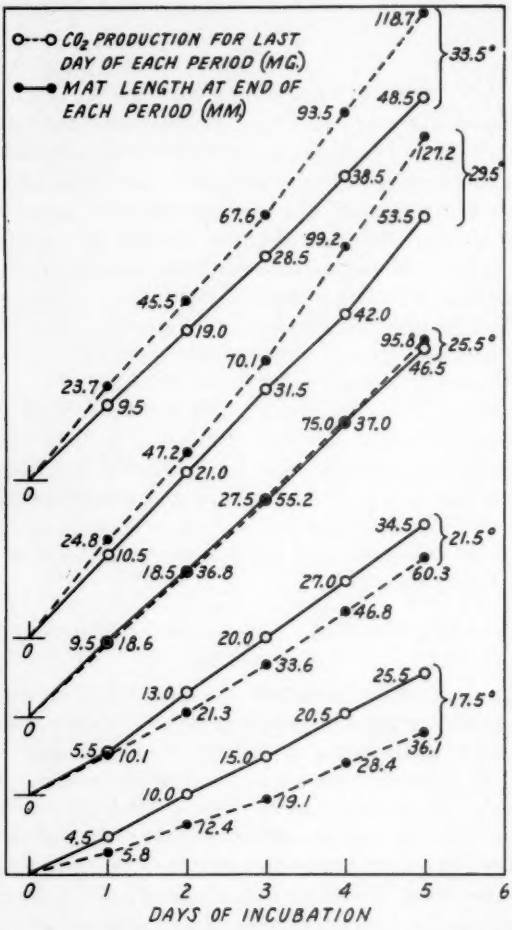

FIG. 6. Time graphs to show relations between length of mycelial mat and daily CO_2 production at each tested temperature.

The maintained daily rates of mat enlargement at the several temperatures are presented by the slopes of the continuous-line graphs of figure 6. The values of these slopes, obtained by dividing each final mat length by 5, are as follows: 17.5° C., 5.1 mm.; 21.5° C., 6.9 mm.; 25.5° C., 9.3 mm.; 29.5° C., 10.7 mm.; and 33.5° C., 9.7 mm. It is seen that the rate of mat enlargement was greater with higher temperature, up to 29.5° C., and that for 33.5° C., it was greater than for 25.5° C. but less than for 29.5° C.

Unlike the rate of mat enlargement, the daily rate of CO_2 production was not maintained throughout the 5-day period at any temperature, for all parts of any mycelial mat were presumably active in producing CO_2 and the mats were continually enlarging. But the mean daily rate, at which the rate of CO_2 production was increased, was nearly maintained for each temperature, as is shown by the fact that the dotted-line graphs of figure 6, although slightly concave upwards, are all very nearly rectilinear. The magnitudes of the acceleration for the several temperatures are the slopes of dotted-line graphs, obtained by dividing by 5 each CO_2-value for the fifth day. These are shown in table I, along with the corresponding mean daily rates of mat enlargement already mentioned, together with the corresponding mean CO_2 accelerations per unit of enlargement.

TABLE I

RATES OF CO_2 PRODUCTION AND MAT GROWTH AT TESTED TEMPERATURES

TEMPERATURE ° C.	MEAN ACCELERATION OF CO_2 MG./DA./DA. (A)	MEAN RATE OF MYCELIAL ENLARGEMENT, MM./DA. (B)	MEAN CO_2 ACCELERATION PER UNIT OF ENLARGEMENT $\left(\frac{A}{B}\right)$
17.5	7.2	5.1	1.4
21.5	12.1	6.9	1.8
25.5	19.2	9.3	2.0
29.5	25.4	10.7	2.4
33.5	23.7	9.7	2.4

Both acceleration values (A) and enlargement rates (B) are seen to be greater with higher temperature, up to 29.5° C.; for 33.5° C. they are less than for 29.5° C. but greater than for 25.5° C.; thus 29.5° C. gave both maximal acceleration of CO_2 production and maximal enlargement rate.

Acceleration of the rate of CO_2 production was clearly related to the continuous enlargement of the mycelial mats, but the ratio values just given above $\left(\frac{A}{B}\right)$ show that the relation of acceleration rate to enlargement rate was not constant but varied with temperature. For each millimeter of mycelial enlargement the daily rate of CO_2 production was increased, during the

5-day period, by about 1.4 mg. per day at the lowest temperature, by about 1.8 mg. per day at 21.5° C., by about 2 mg. per day at 25.5° C., and by about 2.4 mg. per day at the highest two temperatures. That is, the mean rate of CO_2 acceleration per unit of enlargement was progressively greater with higher temperature up to 29.5° C., while for 33.5° C. this ratio value is the same as for 29.5° C. These relations are also brought out graphically in figure 6, where it may be noticed that, in progressing from 17.5° to 29.5° C., the slope of the CO_2 graph is increasingly greater in comparison with the corresponding graph of growth, but that the CO_2 graphs for 29.5° and 33.5° C. are about alike in this respect. The differences here referred to may have been partly due to the temperature relations of the secondary growth, by which the density of the older parts of the mats increased with time; however, further analysis of the data will show that these differences were primarily due to the specific influence of temperature on the respiratory activity of the hyphae. The general relations between temperature and rate of CO_2 production per unit quantity of mycelium will be considered below.

That the nearly straight graphs of daily CO_2 production (dotted lines, fig. 6) are all slightly concave upward appears to indicate that acceleration of the CO_2 rates was not quite maintained throughout the 5-day period but was itself slightly accelerated in some way. The upward curvatures just mentioned may perhaps have been related to a relatively slight but progressively increasing lateral expansion of the mycelial mats, on to the tube walls along the vertical margins of the parallel-sided agar strips, which was regularly observed. Metabolism, as represented by CO_2 production, increased more rapidly, and increasingly so to a slight extent, than was indicated by the upward and downward advance of the mats on to fresh substrate, which advance was essentially constant at any temperature throughout the 5-day period.

General relations between temperature and growth rate

There were naturally some unexplained variations in the recorded growth rates, especially for the first day of the 5-day period. Most of the difficulties arising from these were avoided by omitting the values for the first day when average growth rates for the respective temperatures were computed; these averages represent just the last four days of the 5-day experiment period. Such treatment is of course equivalent to considering the standard adjustment period as though it had actually been 39 hours instead of 15 hours.

The 4-day average daily rates of mycelial advance at each of the five tested temperatures are inscribed on the continuous-line graph of figure 7, A. It appears that the optimal temperature for growth, under these experimental conditions, was not far from 29.5° C. and that 33.5° C. was clearly

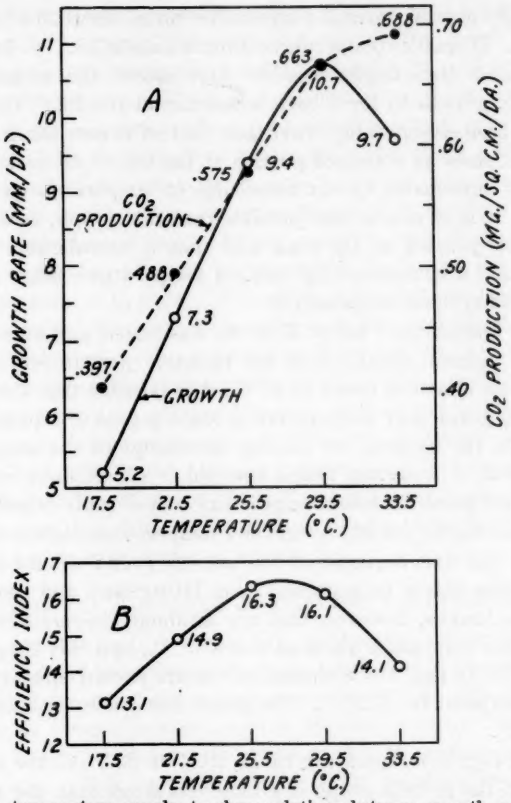

FIG. 7. *A*, temperature graphs to show relations between growth rate and rate of CO_2 production per unit of mycelial area; *B*, temperature graph of growth efficiency with reference to conservation of carbon.

supraoptimal. From what we know of suboptimal temperature relations in general and from the shape of this graph, the lowest temperature tested (17.5° C.) was surely far above the minimal temperature for growth; indeed growth was almost half as rapid at 17.5° as at 29.5° C.

Within the 12-degree temperature range from 17.5° to 29.5° C., the graph of growth rate shows that mycelial advance was reguarly more rapid as the temperature was higher, the difference in rate for a degree of temperature difference being constant throughout all but the upper portion of this suboptimal temperature range. Within the 4-degree range from 25.5° to 29.5° C., the amount of growth increase owing to each degree of temperature increase was progressively less as the temperature was higher, becoming nil for

an exceedingly narrow optimal temperature range about 29.5° C., at the peak of the graph. If smaller temperature intervals about 29.5° C. had been tested, data from such tests might possibly have shown the actual temperature optimum for growth to have been somewhat above 29.5° C., but it could hardly have been considerably lower than that; if it were much lower the true graph should show an S-shaped portion at the left of its peak, which is rendered highly improbable by our knowledge of temperature-growth relations in general. It is of course also possible that this graph, as drawn, may be somewhat too pointed at its peak and that a considerable portion of it should indicate a corresponding optimal temperature range rather than a clearly defined optimal temperature.

Since no temperature below 17.5° C. was tested and since the 4-degree temperature interval employed is too broad to permit very precise study of temperature relations about 17.5° C., it is possible that the first segment of the growth graph may perhaps fail in some degree to represent the actual relations. On the basis of our general knowledge of the temperature relations of growth, it is certain that a reversal in direction of curvature must occur about the point for some temperature considerably below 21.5° C., and such a reversal might possibly occur for a temperature slightly above 17.5° C. In that case the first segment of the growth graph should be slightly S-shaped. It may safely be supposed from HUMPHREY and SIGGER'S (**8**) account of this fungus, however, that the minimal temperature for mycelial growth was not very much above or below 0° C., and this supposition seems to render it likely that the S-shaped curvature should appear below rather than above the point for 17.5° C. The graph was drawn with these considerations in mind.

For the 4-degree temperature range 29.5° to 33.5° C. the general downward slope of the growth graph is a little less steep than the corresponding general upward slope (for the range 25.5° to 29.5° C.). The curvature of the upper portion of the descending segment of the graph may possibly be incorrect to some extent, but it could not be considerably so unless the temperature optimum were significantly above 29.5° C., or a double reversal of curvature (with two S-shaped regions) were introduced between the peak and the fixed point for 33.5° C.

Some preliminary tests indicated that the maximal temperature for growth was about 35° C., which makes it clear that an extrapolation of the growth graph beyond the point for 33.5° C. must descend very steeply indeed, being probably almost vertical throughout a portion of its extent. But the maximal temperature was not ascertained with any great degree of precision. As FAWCETT (**5**) and others have pointed out, the optimal temperature, or the optimal temperature range, is generally more easily ascertained by experiment than is either the minimal or the maximal temperature.

As far as these experimental data show, the optimal temperature for growth apparently remained the same throughout the 5-day experiment period, but the 4-degree temperature intervals used may perhaps have been too broad to bring out any shifting of temperature optimum with progressively longer periods of incubation, if such shifting might be expected with this organism and with the background complex of this study. It is of course also possible that the 5-day experiment period was not long enough to show such shifting if it might occur. It is to be noted that other students of the temperature relations of cultured fungi—for example, FAWCETT (5) and HUMPHREY and SIGGERS (8)—have reported a downward shifting of the temperature optimum with time. That surely could not occur as long as the growth rate for every temperature remained really unchanged throughout the period of incubation, as was reported by FAWCETT (6) for his long-tube cultures and as is also true for the present study. This question of shifting temperature optimum as a culture becomes older deserves attention when intensive studies on temperature-growth relations are to be undertaken. It was discussed by LEHENBAUER (10), in connection with his comprehensive study of the temperature relations of shoot elongation in young maize plants, which are of course very different from these fungus cultures in which there was no evidence of a grand period of growth.

RATES OF CO_2 PRODUCTION IN RELATION TO TEMPERATURE

To obtain average daily rates of CO_2 production corresponding to the average growth rates just discussed, it appeared best to consider only the last two days of the experiment period, as though the adjustment period were taken as 87 hours; in some cases it required a much longer time for the CO_2 rate to become steady than was generally needed in the case of the growth rates. This procedure is logically permissible because both growth rates and rates of CO_2 production per unit of mycelial area were essentially maintained throughout the 5-day experiment period.

To permit satisfactory comparisons among rates of CO_2 production by mycelial mats at different temperatures, each average rate was computed with reference to a standard unit of mycelial area, just as CO_2 rates per unit of body weight are frequently employed in the study of animal and human respiration. The slight lateral growth on to the tube walls was neglected and all mats were taken to be of the same constant width, mat area being consequently considered as proportional to mat length. Each average rate of CO_2 production for the fourth day and also for the fifth was divided by the corresponding average mat length, the latter being half of the sum obtained by adding the mean mat length at the beginning of the day in question to the mean mat length at the end of that day. There were thus secured two series of five values each, a series for each of the two days and in each

series a value for each tested temperature. These two series were finally combined by averaging, so as to give a single series of five values, one for each temperature, to represent both days together. The standard area of mycelial mat, to which these CO_2 rates are referred was first taken as about 20 sq. cm., but each final rate was divided by 20, so as to make it represent approximately a single square centimeter of mat area. This method of computation permits the comparison of CO_2 rates without further reference to the extent of the mycelium that produced the measured CO_2.

The resulting average values for daily CO_2 production per square centimeter of mycelial area at each tested temperature are inscribed on the dotted-line graph of figure 7, *A*. Unity on the scale for CO_2 rates (at the right) corresponds to 16.1 on the scale for growth rates (at the left); *i.e.*, if every CO_2 value, as inscribed, is multiplied by that coefficient (making these rates represent 16.1 sq. cm. of mat instead of 1 sq. cm.) then the resulting reduced CO_2 values may be read directly on the scale shown at the left of the figure. By this method of plotting growth as mm./da. while CO_2 production is plotted as mg./16.1 sq. cm./da., it is seen that the two graphs coincide at the peak of the growth graph and their respective slopes on either side of that point may therefore be compared by inspection.

As in the case of growth, the minimal temperature for CO_2 production is shown to have been far below 17.5° C. It was almost surely much lower for CO_2 production than for growth, for respiration is generally active in organisms at temperatures lower than the minimal temperature for growth.

As might be expected from our general knowledge concerning the temperature relations of respiratory activity, no optimal temperature for CO_2 production is indicated. The average rate of this process was always higher at higher temperatures than at lower ones, throughout the entire 16-degree range considered. This graph of CO_2 rate is like the growth graph in that it is nearly rectilinear for all but the extreme upper part of the temperature range 17.5° to 29.5° C. For a temperature increase of 1° C. the mean increase in CO_2 production for this range was approximately 0.022 mg./sq. cm./da. The 10-degree temperature coefficient (Q_{10}) is about 1.6 for this temperature range; *e.g.*, the CO_2 rate was about 1.6 times as rapid at 27.5° as at 17.5° C.

The general slope of this CO_2 graph for the temperature range 29.5° to 33.5° C. is not so steep as for the range 17.5° to 29.5° C.; its slope for one degree is only 0.006 mg., instead of 0.022 mg. It is thus clear that 29.5° C., which was approximately the optimal temperature for growth, was also a critical temperature for CO_2 production, as has been seen from a study of the time graphs of figure 6. The abrupt change in slope about the point for that temperature may perhaps indicate approach toward a graph maximum, but no temperature higher than 33.5° was thoroughly tested. It is still an interesting question, in general physiology, whether aerobic CO_2 production

is, in general, progressively more rapid at successively higher maintained temperatures, up to or beyond the lethal temperature, or whether there may be generally a critical temperature beyond which still higher maintained temperatures might exert no additional influence on the rate of CO_2 production, or might even give progressively slower rates of this process. In this general connection KOSTYCHEV, as translated by LYON (**9**, p. 172) says: "The amount of carbon dioxide given off increases regularly with gradual increase of temperature, finally reaches a limiting value and remains at that level until the plant dies from the high temperature." This statement properly refers to experiments in which temperature rises with the lapse of time, but the same general relation holds when maintained temperatures are considered, as in the present study.

COMPARISON OF GENERAL TEMPERATURE RELATIONS OF GROWTH WITH THOSE OF CO_2 PRODUCTION

As has been noted, within the 12-degree temperature range of 17.5° to 29.5° C., both growth and CO_2 production were regularly more rapid at higher temperatures than at lower ones and the two graphs of figure 7, *A* are somewhat alike for that range. But of course the two graphs show no similarity beyond the point for 29.5° C., which represents the optimal temperature for growth and a critical temperature for CO_2 production.

Some quantitative relations between mycelial growth and CO_2 production in these experiments may be brought out by means of the quotients obtained by dividing each average growth value by the CO_2 value for the same temperature. The resulting quotients may be called (**12**) coefficients of growth efficiency with reference to carbon conservation. Each efficiency coefficient represents, for the specified temperature, the average daily rate of mycelial growth per milligram of CO_2 production per square centimeter of mycelium. Since growth is inevitably accompanied by CO_2 production and consequent loss of carbon, the efficiency of growth, as far as carbon conservation is concerned, is greater as the value of this efficiency coefficient is higher. Loss of carbon is relatively more rapid with low efficiency, and conversely.

The five efficiency coefficients derived from the values inscribed on the graphs of figure 7, *A* are, for the several tested temperatures: 17.5° C., 13.1 (*i.e.*, 5.2/0.397); 21.5° C., 14.9; 25.5° C., 16.3; 29.5° C., 16.1; and 33.5° C., 14.1. The cultures at 25.5° and at 29.5° C. showed high efficiency while those at 17.5° and at 33.5° C. showed low efficiency, the coefficient for 21.5° C. being intermediate in magnitude. This series of efficiency coefficients is shown graphically in figure 7, *B*. Further discussion of this concept of growth efficiency with reference to carbon conservation is deferred until data for aerial environments other than ordinary air become available.

Summary

1. In this paper are reported results of an experimental study concerning the influence of temperature and length of incubation period on vegetative growth and carbon dioxide production in continuously aerated cultures of a strain of a wood-destroying fungus, *Polystictus versicolor* (Linn.) Fr., growing for five days on malt-agar substrate in darkness. Five different maintained temperatures were employed: 17.5°, 21.5°, 25.5°, 29.5° and 33.5° C. Average daily rates of growth and CO_2 production are presented, also rates of CO_2 production per unit of mycelium.

2. A full account is given, with diagrams and photographs, of the special methods and devices employed, including a new tube for cultures with continuous gas flow, arrangements for controlling the flow of gas (at the regular rate of 15 liters a day), temperature control, apparatus for collection and measuring the CO_2 produced, preparation of the agar strips that served as substrate and preliminary treatment of the cultures.

3. Mycelial growth was found to proceed both upward and downward at the same rate, showing no gravitational influence at any tested temperature. The rate of growth at each tested temperature was maintained throughout the 5-day experiment period. The average daily rate of growth was more rapid as temperature was higher, from 17.5° to 29.5° C., the latter temperature being about optimal for growth. The minimal temperature for growth, which was far below the lowest temperature tested, was probably not far from zero. The highest temperature tested in the experimental series (33.5° C.) was clearly supraoptimal for growth, the maximal temperature being indicated as about 35° C. There is no evidence of a downward shifting of the optimal temperature with increasing length of the period of incubation.

4. The rate of CO_2 production increased regularly at every tested temperature, throughout the 5-day period, as the mats elongated and their older parts tended to mature. But the rate of increase in the rate of CO_2 production, or acceleration, although shown to have been itself very slightly increased toward the end of the period, perhaps because of some lateral spread of the mats on the walls of the culture tubes, was, on the whole, essentially maintained at each temperature. This acceleration was greater as temperature was higher, up to 29.5° C., while at 33.5° C. it was slightly less than at 29.5° C. but greater than 25.5° C. Thus 29.5° C. gave both maximal acceleration of CO_2 production and maximal enlargement rate. The value of the ratio of mean rate of acceleration to mean rate of enlargement was also greater with higher temperature, up to 29.5° C., but this ratio value was the same for 33.5° as for 29.5° C.

5. For CO_2 production per unit of mycelial mat no minimal, optimal, or maximal temperature was shown; the minimal temperature was apparently far below 17.5° C. and probably much below the minimal temperature for

growth. Throughout the entire range of tested temperatures the rate of CO_2 production was more rapid as temperature was higher and the 10-degree temperature coefficient of this process was about 1.6 for the temperature range from 17.5° to 29.5° C. But this coefficient was smaller for the higher temperature range from 29.5° to 33.5° C., indicating that the optimal temperature for growth (29.5° C.) was also a critical temperature for CO_2 production per unit of mycelium. The two processes were much alike in regard to their temperature relations within the range 17.5° to 29.5° C., but above 29.5° C. they differed greatly.

6. A coefficient of growth efficiency with reference to conservation of carbon is introduced, being the quotient obtained by dividing each average growth rate by the corresponding average rate of CO_2 production per unit of mycelium. Its value for any temperature represents the average daily rate of mycelial advance per milligram of CO_2 production per unit of mycelial area. Vegetative growth proceeded with relatively least loss of carbon (as CO_2) at the medium temperatures, 25.5° and 29.5° C., but loss of carbon was relatively great for 17.5° and 33.5° C.

The writer gratefully acknowledges many helpful suggestions received from Dr. CARL HARTLEY and other members of the U. S. Bureau of Plant Industry in the inception of this study, and for a great deal of help received from Prof. BURTON E. LIVINGSTON, of Johns Hopkins University, throughout the progress of these experiments and in the preparation of this paper.

JOHNS HOPKINS UNIVERSITY
BALTIMORE, MARYLAND

LITERATURE CITED

1. DE BOER, S. R. Respiration of *Phycomyces*. Rec. Trav. Bot. Néerland. **25**: 117–239. 1928.
2. BONNIER, M. G., and MANGIN, L. Recherches sur la respiration et la transpiration des champignons. Ann. Sci. Nat. Bot. VI, **17**: 210–305. 1884.
3. CHASTUKIN, V. IÂ. Issledovaniiâ po fiziologii gribov. Materialy po Mikol. i Fitopath. Leningrad **8**: 3–55. 1929.
4. CHRISTIANSEN, J. E., VEIHMEYER, F. J., and GIVAN, C. V. Effect of variations in temperature on the operation of the instantaneous reading atmometer. Ecol. **11**: 161–169. 1930.
5. FAWCETT, H. S. Temperature relations of growth in certain parasitic fungi. Univ. California Publ. Agr. Sci. **4**: 183–232. 1921.
6. ————. Maintained growth rates in fungus cultures of long duration. Ann. Appl. Biol. **12**: 191–198. 1925.
7. GREGORY, F. G. A simple device for regulating gas flow. Ann. Bot. **47**: 427–428. 1933.

8. HUMPHREY, C. J., and SIGGERS, P. V. Temperature relations of wood destroying fungi. Jour. Agr. Res. **47**: 997–1008. 1933.
9. KOSTYCHEV, S. Plant respiration. Trans. by CHARLES J. LYON. Philadelphia. 1927.
10. LEHENBAUER, P. A. Growth of maize seedlings in relation to temperature. Physiol. Res. **1**: 247–288. 1914.
11. LIVINGSTON, B. E., and FAWCETT, H. S. A battery of chambers with different automatically maintained temperatures. Phytopath. **10**: 336–340. 1920.
12. ————, and MACK, W. B. A coefficient of growth efficiency with reference to carbon conservation. Amer. Jour. Bot. In press.
13. MACK, W. B. The relation of temperature and the partial pressure of oxygen to respiration and growth in germinating wheat. Plant Physiol. **5**: 1–68. 1930.
14. ————, and LIVINGSTON, B. E. Relation of oxygen pressure and temperature to the influence of ethylene on carbon-dioxide production and on shoot elongation in very young wheat seedlings. Bot. Gaz. **94**: 625–687. 1933.
15. SCHEFFER, T. C. A tube for culturing fungi. Science n.s. **82**: 467–468. 1935.
16. WASSINK, E. C. Begrenzende Bedingungen bei der Atmung von *Phycomyces*. Rec. Trav. Bot. Néerland. **31**: 583–690. 1934.
17. ZINZADZE, CH. New arrangement for regulating flow of liquid into a culture vessel. Science n.s. **81**: 540–542. 1935.

RESPIRATION AND METABOLISM IN ETIOLATED WHEAT SEEDLINGS AS INFLUENCED BY PHOSPHORUS NUTRITION

WINSTON W. JONES

(WITH TEN FIGURES)

Introduction

Phosphorus plays an important rôle in the process of respiration (**12, 13, 29**). It forms, in the presence of sugars, mono- and di-hexosephosphoric acid esters (**8, 27**), which are then broken down in the respiratory process. Under certain conditions it may form with sucrose a sucrose phosphoric acid ester (**19**). It is also known that plants starved for phosphorus differ considerably in their chemical composition from those not thus starved. MACGILLIVRAY (**15**) has pointed out that tomato plants starved for phosphorus show a decrease in coagulable nitrogen, and an increase in total nitrogen. There is also a marked increase in percentage of sugar. KRAYBILL (**10**) also showed that when phosphate is limiting in tomato plants nitrates as well as carbohydrates accumulate. According to ECKERSON (**5**) the lack of phosphorus causes a breakdown of the reducase activity of the plant, which is then followed by an accumulation of nitrates, sugars, and starch, and results in an essentially nitrogen-starved plant. It is desirable then to know the relation between respiration and the chemical composition of plants deficient in phosphorus as compared with plants not deficient in phosphorus.

In view of these facts and because phosphorus very often becomes limiting in agricultural soils the present investigation was undertaken. All of the chemical data cited above were obtained with green plants and after phosphorus starvation had become rather severe. It was the desire of the writer to determine some of the early symptoms of phosphorus starvation as shown by internal composition, as well as to measure the respiration during this period, and to relate any differences in composition to respiration, if possible. For these purposes large green plants are not suited; hence, etiolated seedlings with a considerable reserve of carbohydrates were used. Wheat was chosen as the most uniform plant at hand. Since LYON had obtained an increase in respiration owing to increased phosphate supply, the respiration behavior of wheat was reinvestigated, and, at the same time, the chemical changes caused by differences in phosphorus nutrition during early development were determined. It will be noted that the differences obtained in this work with different phosphorus treatments are small, as would be expected, since the seeds contain some phosphorus. The results, however, are in most cases consistent.

Materials and culture methods

Choice of seed

The seed used for this work had to meet two conditions: first, it must contain a relatively large stored food supply; and second, it must be small enough that a representative sample would not be unduly bulky. Wheat seemed to meet these requirements very well. A third desideratum was a low phosphorus content, but, as this quality depends somewhat on the nature of the soil in which the wheat is grown, a crop was grown to maturity in the greenhouse in a phosphorus-deficient nutrient solution. This crop yielded the seeds for the first part of the experiment, but owing to the small quantity of seeds obtained, field-grown seeds were used for the chemical studies. These field-grown seeds show respiratory responses to phosphate additions similar to those shown by the greenhouse-grown seeds, although the differences are not so marked. The seeds used were *Tricitum vulgare* Vill. vars. Marquis and Poso, and *T. durum* Desf. var. Kubanka.

Greenhouse plants

Marquis wheat was grown in the greenhouse in 2-gallon stone crocks filled with quartz sand. One set received each second day a nutrient solution containing phosphorus and the other set a solution lacking phosphorus. Distilled water was added when needed, and the pots were flushed once each week with distilled water. At the end of 23 days the plants without phosphorus were making such little growth that one application of the nutrient solution containing phosphorus was made. When the plants were mature the seeds were harvested and used in the respiration studies. They were placed in the respirometer chambers, with or without phosphorus, as described later under chemical methods. The nutrient solution used in each case throughout this work was made from 0.5 M stock solution the composition of which was as follows:

+P		−P	
Salt	Concentration	Salt	Concentration
KH_2PO_4	0.005 M	KCl	0.005 M
$Ca(NO_3)_2$	0.005 M	$Ca(NO_3)_2$	0.005 M
$MgSO_4$	0.005 M	$MgSO_4$	0.005 M

In each case the total concentration of the nutrient solution was 0.015 M. It was adjusted to a pH of about 5.4.

Field seed

Wheat seeds of the variety Poso were obtained from the United States Department of Agriculture Experiment Station at Davis, California, and the Kubanka from the North Dakota Agricultural Experiment Station. The

Marquis spring wheat was in the laboratory. In table I are shown the protein, starch, and phosphorus content of the seeds of these three varieties.

TABLE I

COMPOSITION OF FIELD-GROWN SEEDS IN PERCENTAGE OF DRY WEIGHT

WHEAT	YEAR OF CROP	WT. OF 100 SEEDS	PROTEIN	STARCH	PHOSPHORUS
		gm.	%	%	%
Marquis	1933	2.34	17.42	39.87	0.202
Poso	1935	2.53	11.35	42.10	0.050
Kubanka	1933	3.09	18.39	41.68	0.183

For chemical analysis, 25 gm. of seeds were sterilized in 0.25 per cent. uspulun for 20 minutes. Two sets of seeds were then germinated on cellucotton. One set was moistened with a nutrient solution with phosphorus, the other set with a nutrient solution without phosphorus. After 24 hours' germination they were transferred to wire netting supported over a 4-gallon stone crock containing the nutrient solution. The nutrient solution was kept constantly stirred by means of a flowing air stream. They were grown for 7 days in a dark room at 27° C. A complete change of the nutrient solution was made each 48 hours. At the end of the 7-day period the plants were harvested and treated as described under chemical methods.

Chemical methods

RESPIRATION

DETERMINATION OF CARBON DIOXIDE.—The conductivity method was used for the determination of carbon dioxide. The absorption towers were similiar in construction to those described by THOMAS (**30**). The towers were standardized and used as described by MITCHELL (**17**). The LiOH was 0.2 N strength. The respiratory flasks were 125-ml. Erlenmeyer flasks blackened on the outside to exclude light. The flasks containing the respiring seeds were placed, along with the absorption towers, in a constant temperature bath. The variation in temperature was $\pm$ 0.001° C. Each respiration experiment was run in duplicate for 7 days. The procedure was as follows: One hundred seeds were sterilized in 0.25 per cent. uspulun for 20 minutes. The uspulun was then washed off with distilled water and the seeds were placed on moist cellucotton in the respiratory flasks. The cellucotton was moistened with the nutrient solution, either with or without phosphorus. The flasks were then placed in the water bath and the CO_2-free air stream turned on. One hour was allowed for the temperature adjustment. At the

end of this 1-hour period the air stream was turned through the absorption towers and the CO_2 absorbed for a 5-hour period, when the air was turned out of the absorption towers and the absorbed CO_2 determined. At the beginning of the second 24-hour period the CO_2 was again absorbed for a 5-hour period, so that at the end of 7 days the CO_2 had been determined for 7 five-hour periods.

Fractionation and extraction

At the end of the 7-day period the plants grown in the dark room were harvested and separated into tops, roots, and seed-remains. The samples taken were covered with hot 95 per cent. alcohol, enough being used so that the final concentration was 80 per cent. The samples were extracted as soon as possible with hot 80 per cent. alcohol, the alcohol being boiled each time on the steam bath for 15 minutes. The extract was allowed to cool to room temperature before filtering. Seven extractions were made.

Carbohydrate fractions.—For sugars, an aliquot of the alcoholic extract was evaporated down and water added, and the water solution cleared with neutral lead acetate and potassium oxalate, as given by the Committee on Methods (**2**). The reducing sugar was then determined on this cleared solution by the bicarbonate modification of the Tompsett method as adapted and described by Phillips (**24**). The residue was dried and ground so as to pass through an 80-mesh sieve and again dried. Starch was determined on this residue as described by Niemann *et al.* (**20**), and the reducing power of the resulting sugar determined as above for reducing sugar. No sucrose was found and starch was present only in the seed-remains.

Nitrogen fractions.—Total soluble nitrogen in the extract was determined by the reduced iron method of Pucher, Leavenworth, and Vickery (**26**) modified and adapted to the micro-Kjeldahl method described by Pregl (**25**). One-ml. aliquots of the alcoholic extract were placed in the micro-Kjeldahl digestion flasks and 0.29 ml. of 1:1 H_2SO_4 and 0.086 gm. reduced iron added to each. The flasks were then shaken for 10 minutes and boiled over a low flame for 5 minutes. The contents were cooled and 0.86 ml. of concentrated H_2SO_4 and a knife point of a mixture of K_2SO_4 and $CuSO_4$ (1:3) were added to each. The digestion and distillation was then completed as described by Pregl. Pregl's method was also used for the determination of the insoluble nitrogen of the residue.

Phosphorus fractions.—Soluble phosphorus was determined on 10-ml. aliquots of the alcoholic extract. The aliquot was evaporated almost to dryness, then digested, and phosphorus determined by the method described by Cockefair (**1**). The phosphorus in the residue was determined on 100-mg. samples by the same method.

Data

Respiration of greenhouse-grown seeds

The data for this paper will be presented in two parts: first, those dealing with the respiration of the greenhouse-grown seeds; and second, those dealing with the respiration and chemical composition of the field-grown seeds. Table II shows the composition of the greenhouse-grown seeds.

TABLE II

Effect of phosphorus deficiency on weight and chemical composition of Marquis wheat seeds

Nutrient	No. of seeds from 22 pots	Wt. of 100 seeds	Protein	Starch	Phosphorus
			Dry wt. basis	Dry wt. basis	Dry wt. basis
		gm.	%	%	%
Deficient in P	1300	1.4646	15.80	37.40	0.444
Complete nutrient	1563	1.7101	15.02	44.60	0.688

Table II shows that the phosphorus-deficient plants produced fewer and smaller seeds, and that these seeds were slightly higher in protein but lower in starch and phosphorus than those seeds from the plants grown in a complete nutrient solution. Although the seeds from the phosphorus-deficient plants were much lower in phosphorus, yet they contained considerable of this element, this phosphorus having come from the original seed and from the one application of the phosphate-containing nutrient solution. In contrast with the data given in table II, Gericke (6) obtained much more growth, earlier maturity, and greater fresh weight with wheat when grown

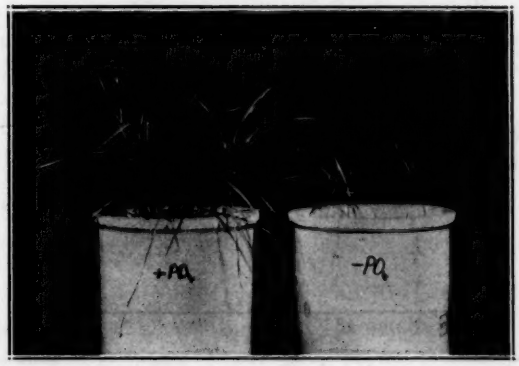

Fig. 1. Marquis wheat 28 days old grown in cultures with and without phosphorus.

in a nutrient solution lacking phosphate after the first 4 weeks of growth with phosphate.

Figure 1 shows the condition of Marquis spring wheat at 28 days of age in sand culture with and without phosphorus. These – P plants did not

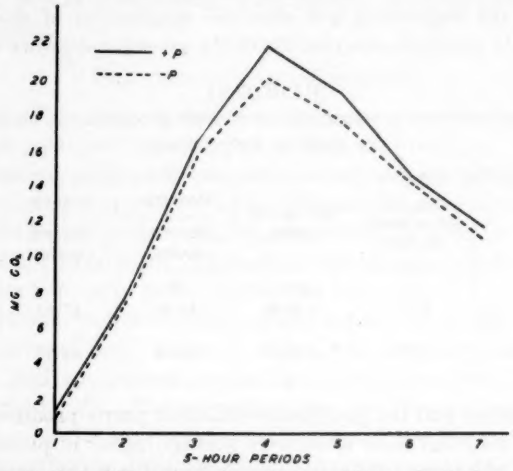

FIG. 2. Respiration of Marquis spring wheat seedlings. Seeds from plants not deficient in phosphorus. Data for graphs are in milligrams of CO_2/5-hour period/gm. of air-dry seeds.

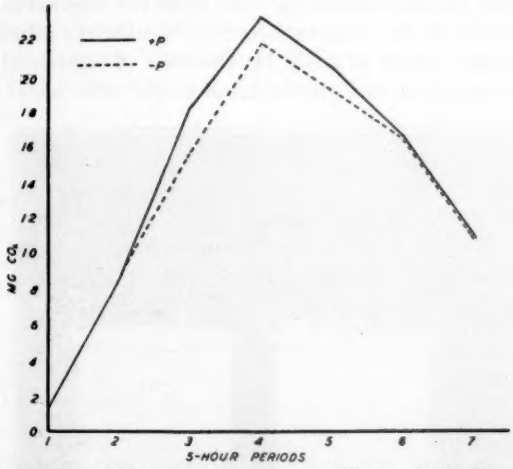

FIG. 3. Respiration of Marquis spring wheat seedlings. Seeds from plants deficient in phosphorus. Data for graphs are in milligrams of CO_2/5-hour period/gm. of air-dry seeds.

receive phosphorus on the twenty-third day as did the ones from which the seeds were obtained. The plants shown died, in the case of the – P plants, a few days after the photograph was taken.

Table II gives the CO_2 produced per gram of air-dry seeds for the first 7 days after germination. The figures represent the amount in milligrams of CO_2 produced per gram per 5-hour period. The data for the seeds from deficient and non-deficient phosphorus plants when grown in solutions with and without phosphorus are given. This is represented graphically in figures 2 and 3.

TABLE III

CO_2/5-HOUR PERIOD/GM. OF AIR-DRY SEEDS ON EACH OF THE FIRST 7 DAYS AFTER GERMINATION FOR MARQUIS WHEAT SEEDS DEFICIENT AND NOT DEFICIENT IN PHOSPHORUS

TREATMENT	MG. CO_2/5-HOURS/GM.						
	1	2	3	4	5	6	7
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
+ P seeds in – P nutrient	0.58	7.41	15.71	20.05	17.64	14.23	11.11
+ P seeds in + P nutrient	1.10	7.54	16.42	21.80	19.21	14.70	11.80
- P seeds in – P nutrient	1.16	8.46	15.75	21.86	19.33	16.46	11.06
- P seeds in + P nutrient	1.16	8.46	18.20	23.33	20.60	16.66	11.09

When phosphorus is lacking in the nutrient solution of Marquis wheat seedlings, growth is greatly retarded, as shown in figure 1. If phosphorus is entirely lacking in the nutrient solution the wheat seedlings die after about 5 weeks. The phosphorus contained in the old seeds is not sufficient to carry the plants to maturity. When a small amount of phosphorus is supplied, so that phosphorus is still limiting but not lacking, the plants grow to maturity, but the total yield and weight per seed is much less for the phosphorus-deficient plants than for the plants not deficient in phosphorus. Furthermore, the chemical composition of the seeds from the phosphorus-deficient plants is different from that of the seeds from plants not deficient in phosphorus as shown in table II.

From table III and figures 2 and 3 it is shown that when phosphorus is deficient the amount of CO_2 evolved in respiration is reduced.

RESPIRATION OF FIELD-GROWN SEEDS

Table I shows the main constituents of the field-grown seeds. It will be noted that the Marquis spring wheat and Kubanka are both high in protein, and the Poso is low in protein. However, the protein content may vary

widely in a given variety as shown by McGinnis (18) and in table II, depending on the conditions under which the seeds were produced. It is interesting to compare the responses of the various wheats to phosphate. Table IV shows the CO_2 produced by each of the three wheats for a period of 7 days when grown in a complete nutrient solution and also when grown in a solution lacking phosphorus. The figures represent CO_2/5-hour period/gm. as before. These data are shown graphically in figures 4, 5, and 6.

TABLE IV

CO_2/5-HOUR PERIOD/GM. OF AIR-DRY SEEDS ON EACH OF 7 DAYS FOR MARQUIS, POSO, AND KUBANKA WHEAT SEEDLINGS

WHEAT	TREATMENT	MG. CO_2/5-HOUR PERIOD/GM.						
		1	2	3	4	5	6	7
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Marquis	+P nutrient	0.96	6.39	14.45	20.06	21.00	18.85	17.56
	−P nutrient	0.96	5.98	13.85	18.65	19.79	17.58	15.30
Poso	+P nutrient	0.73	2.65	7.68	15.01	18.78	17.72	16.95
	−P nutrient	0.69	2.37	6.09	12.42	17.20	16.91	15.95
Kubanka	+P nutrient	1.38	4.95	8.84	14.50	16.76	15.69	14.35
	−P nutrient	0.55	4.40	8.58	13.86	16.11	14.99	13.04

It is to be noted that, except for the first 5-hour period of the Marquis wheat, the CO_2 production of the plants growing in a complete nutrient solu-

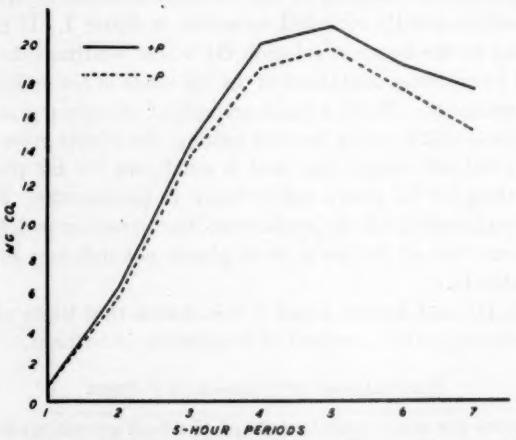

FIG. 4. Respiration of Marquis spring wheat seedlings, with and without phosphorus. Seeds from field-grown plants. Data for graphs are in milligrams of CO_2/5-hour period/gm. of air-dry seeds.

tion is always greater than that of the plants in a solution lacking phosphorus. The respiration of both sets of plants increases to a maxmium at the fifth 5-hour period, after which there is a slight decrease. At the end of the 7-day period other plants grown similarly were harvested and separated into tops, roots, and seed-remains. These were then analysed for reducing sugars, sucrose (none was found), starch, soluble nitrogen, insoluble nitrogen, soluble phosphorus, and insoluble phosphorus. These data are given in table V.

TABLE V

CHEMICAL COMPOSITION OF MARQUIS, POSO, AND KUBANKA WHEATS AFTER 7 DAYS GERMINATION IN THE DARK WITH AND WITHOUT PHOSPHORUS. RESULTS IN PERCENTAGE OF DRY WEIGHT

VARIETY OF WHEAT	FRACTION	+P			−P		
		TOPS	ROOTS	SEED	TOPS	ROOTS	SEED
		%	%	%	%	%	%
Marquis	Percentage of H_2O	91.41	93.49	81.08	90.69	93.27	80.35
	Reducing sugars	6.43	8.11	7.14	8.28	6.40	9.66
	Starch	None	None	15.01	None	None	12.68
	Soluble N	3.04	2.41	0.50	3.32	3.56	0.64
	Insoluble N	6.22	4.54	2.21	5.87	3.55	2.14
	Total N	9.26	6.95	2.71	9.19	7.11	2.78
	Soluble P	0.59	0.83	0.054	0.039	Trace	0.046
	Insoluble P	0.446	0.347	0.220	0.308	0.160	0.068
	Total P	1.036	1.177	0.274	0.347	0.160	0.114
Poso	Percentage of H_2O	91.85	93.76	79.78	91.92	93.62	78.09
	Reducing sugars	7.64	5.93	8.15	10.52	7.67	9.31
	Starch	None	None	13.75	None	None	16.70
	Soluble N	3.13	2.98	1.14	2.49	3.95	1.30
	Insoluble N	6.08	4.39	2.13	5.80	3.90	2.17
	Total N	9.21	7.37	3.27	8.29	7.85	3.47
	Soluble P	Trace	0.01	Trace	Trace	Trace	Trace
	Insoluble P	0.59	0.75	0.60	0.36	0.42	0.58
	Total P	0.59	0.76	0.60	0.36	0.42	0.58
Kubanka	Percentage of H_2O	91.68	92.56	85.25	91.09	92.72	76.70
	Reducing sugars	4.77	5.66	11.21	5.37	5.39	8.14
	Starch	None	None	14.95	None	None	18.74
	Soluble N	3.04	1.75	0.66	3.22	2.89	0.82
	Insoluble N	5.84	4.03	2.35	5.56	3.34	2.27
	Total N	8.88	5.78	3.01	8.78	6.23	3.09
	Soluble P	0.24	1.63	0.07	0.04	Trace	Trace
	Insoluble P	0.75	0.95	0.52	0.53	0.40	0.48
	Total P	0.99	2.58	0.59	0.57	0.40	0.48

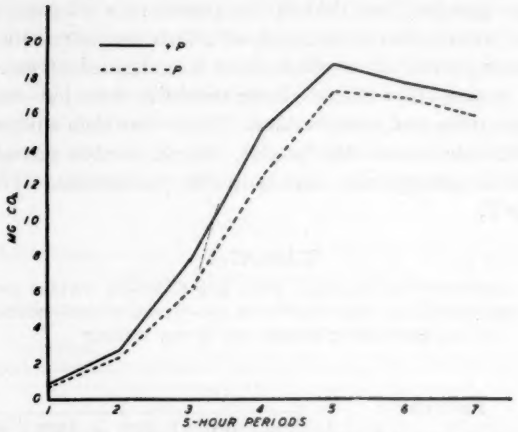

FIG. 5. Respiration of Poso wheat seedlings, with and without phosphorus. Seeds from field-grown plants. Data for graphs are in milligrams of CO_2/5-hour period/gm. of air-dry seeds.

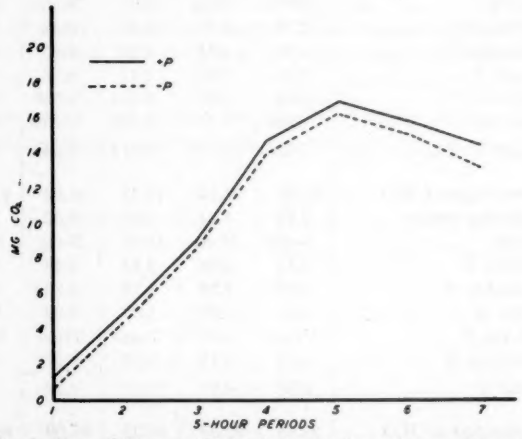

FIG. 6. Respiration of Durum (Kubanka) wheat seedlings, with and without phosphorus. Seeds from field-grown plants. Data for graphs are in milligrams of CO_2/5-hour period/gm. of air-dry seeds.

Table V and figures 7, 8, 9, and 10 show that there is a very marked difference between plants grown on a minus-phosphorus nutrient solution and on a complete nutrient solution. First, the plants grown on the minus-phosphorus nutrient solution have a lower insoluble nitrogen content than those grown with phosphorus. Secondly, the –P plants have, in most cases, a

higher soluble nitrogen content than the + P plants. Furthermore, the reducing sugar content is, in most cases, higher in the – P plants, and yet the respiration of the – P plants is less than the + P plants (figs. 4, 5, 6). The difference in phosphorus content, especially soluble phosphorus, is very marked, as would be expected. In the plants lacking phosphorus in the nutrient solution there was only a trace of soluble phosphorus, while in the + P plants the soluble phosphorus was as high as 1.63 per cent. of the dry weight in one case.

Discussion

In the metabolism of plants phosphorus has several important functions (**1**). WOODWARD (**32**), as long ago as 1699, recognized the importance of phosphorus in the formation of plant bodies. Phosphorus occurs in the plant both in an inorganic form and in an organic form. VON OHLEN (**31**) shows that in germination there is a continual change of the organic phosphorus of the seed to the inorganic form which moves to the growing points where it again changes to the organic form. It may occur organically bound as nucleic acid (**14**), lecithin, cephalin, sphingomyelin (**11**), hexosediphosphate (**8**), hexosemonophosphate (**27**), and sucrosephosphate (**19**).

The present study concerns itself with the relation of phosphorus to protein and carbohydrate metabolism, and its relation to respiration.

RESPIRATION

That phosphorus is important in the process of respiration is shown by the work of HARDEN and YOUNG (**8**) on the fermentation of glucose by yeast-juice. These workers obtained large increases in the rate of fermentation and in the total amount of fermentation when they added a phosphate salt to the fermenting mixture of glucose and yeast-juice. The phosphorus forms with the glucose a hexosediphosphate which is then split up yielding an easily oxidized sugar and liberating the phosphorus. The phosphorus is then free to unite with more glucose. GOTTSCHALK (**7**), studying the action of a co-zymase in connection with hexose phosphate, concludes that fermentation, caused by yeast, results in splitting off from glycogen small fractions of alpha, beta, or balanced glucose which is phosphorylated, perhaps by the aid of co-zymase. From this phosphorylated compound is liberated a readily decomposable form of sugar which is susceptible to seizure by destructive enzymes. Such enzymes occur in plants (**16**) and could easily oxidize, or dehydrogenate, such unstable compounds as the hexose liberated from the hexosediphosphate. The rôle of phosphorus in respiration seems to be to form unstable substrates for the respiratory enzymes which attack these substrates and by their action liberate energy. For this purpose only small quantities of phosphorus are needed as it is not permanently bound. Other compounds, as

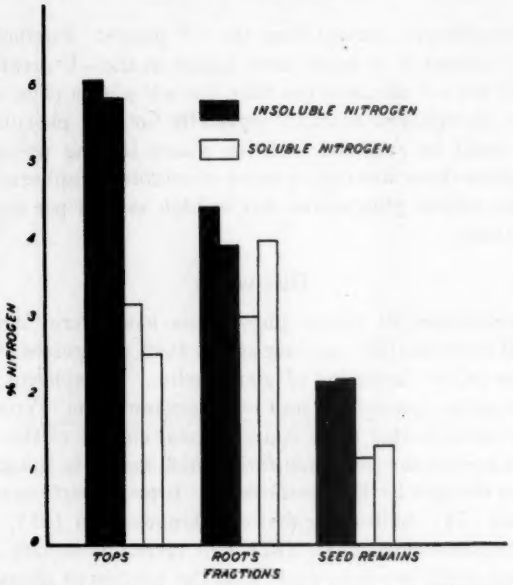

FIG. 7. Nitrogen content of 7-day-old Marquis wheat seedlings. Left bar, with phosphorus; right bar, without phosphorus.

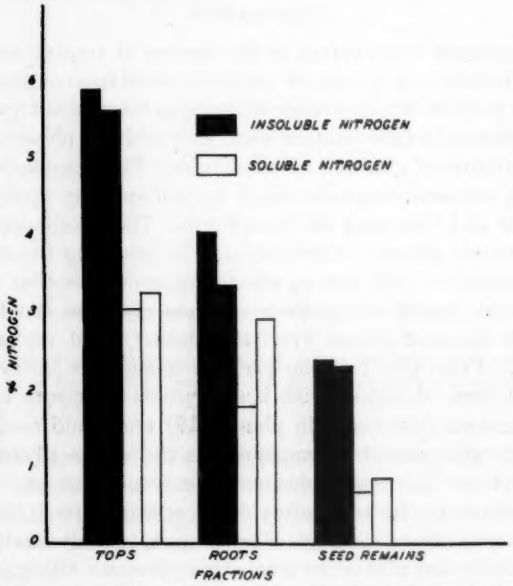

FIG. 8. Nitrogen content of 7-day-old Poso wheat seedlings. Left bar, with phosphorus; right bar, without phosphorus.

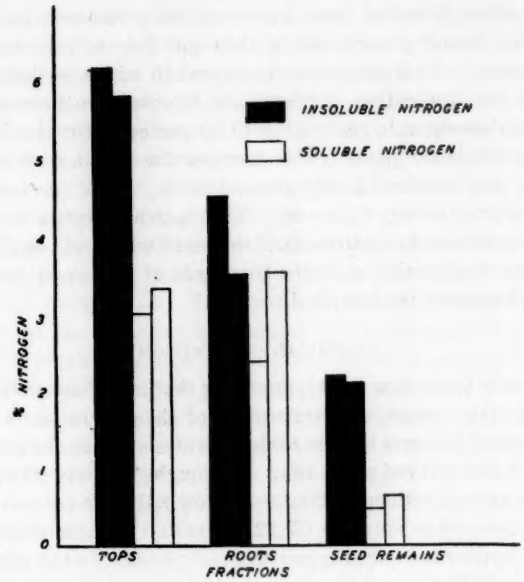

FIG. 9. Nitrogen content of 7-day-old Kubanka wheat seedlings. Left bar, with phosphorus; right bar, without phosphorus.

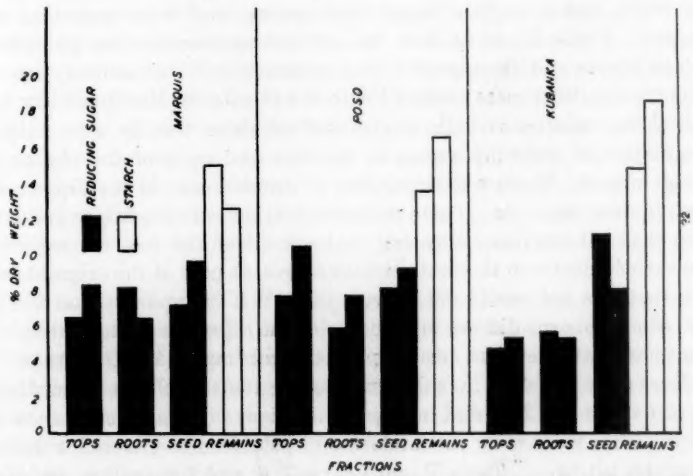

FIG. 10. Carbohydrate content of 7-day-old wheat seedlings. Left bar, with phosphorus; right bar, without phosphorus.

pointed out above, however, form a more or less permanent union with phosphorus. This bound phosphorus is then not free to take part in the respiratory process. In *Elodea canadensis* and in wheat seedlings LYON (**12**) was able, by the application of phosphate, to obtain an increase in the production of carbon dioxide amounting to 55 per cent. for the former and 35 per cent. for the latter plant. This increase for wheat seedlings was much greater than was obtained in the present work. Here the increase was, in most cases, approximately 6 per cent. This increase bears a very direct relation to certain chemical constituents of the plant which will be discussed later. LYON gives no explanation as to the treatment of the wheat seedlings except to say that phosphate was supplied.

CHEMICAL COMPOSITION

According to ECKERSON (**4, 5**), anything that interferes with the reducase activity of a plant causes an interruption of the general metabolism of the plant. The plant becomes high in carbohydrates and has the general appearance of a nitrogen-starved plant (**9**). Although there are plenty of nitrates present, they are not reduced owing to the low reducase content of the plant. Also plants deficient in sulphur (**3, 22**), calcium (**21**), or phosphorus (**4, 5, 15**) have the appearance of nitrogen-starved plants. In the absence of these elements the reducase content is low.

In the 7-day-old wheat seedlings, the plants in the complete nutrient solution as compared with those in the minus-phosphate solution were somewhat taller, had a slightly larger root system, and were somewhat more succulent. Table V shows that the differences between the phosphorus-deficient plants and those grown in a complete nutrient solution are very similiar to the differences obtained with the tomato by MACGILLIVRAY (**15**). In all three varieties of both species studied there was, in most cases, an accumulation of reducing sugars in the tops and roots of the phosphorus-deficient plants. There was no sucrose or starch found in the tops or roots of the plants at any time. There was considerable reducing sugar and starch left in the seed-remains indicating that not all of the reserves were used. These carbohydrates in the seed-remains represent part of the original stored starch that was not moved out. From table V it is apparent that the deficiency of phosphorus did not interfere with the digestion of the starch.

In phosphorus-deficient tomato plants, according to MACGILLIVRAY (**15**) and ECKERSON (**4, 5**); in sulphur-deficient tomato plants, according to NIGHTINGALE *et al.* (**22**); and in sulphur-deficient soy bean plants, according to EATON (**3**), there is an accumulation of soluble nitrogen and a decrease in insoluble nitrogen. Table V and figures 7, 8, and 9 show that this is also true of phosphorus-deficient wheat seedlings. The percentage of insoluble nitrogen was always greater in the seedlings grown in a complete nutrient

solution, while the soluble nitrogen was greater in the phosphorus-deficient plants. The lack of phosphorus probably did not interfere with the hydrolysis of the proteins stored in the seed, but in the resynthesis of these proteins and in the formation of new proteins from the nitrates of the nutrient solution the phosphorus was apparently limiting. This limiting action was probably of a dual nature: (1) those proteins containing phosphorus could not be synthesized, and (2) the reduction of nitrates was hindered owing to the low reducase content (5) of the plants.

Table V shows that the plants from a complete nutrient solution contain more than twice as much total phosphorus as the plants from a nutrient solution lacking phosphorus. This was expected, but the distribution of the phosphorus in the insoluble and soluble forms offers a still greater point of contrast. Except in the case of the Poso, in the plants grown on a complete nutrient solution about 50 per cent. of the total phosphorus was in the soluble form, while in the plants grown on the phosphorus-deficient nutrient solution only about 5 per cent. or less of the total phosphorus was in the soluble form. The soluble phosphorus determinations were run on the alcoholic extract, and, according to LEVENE (11), all of the phosphatides are extracted by hot alcohol. If this is true then there was only a very small trace of phosphatides in the phosphorus-deficient plants.

RELATION BETWEEN RESPIRATION AND CHEMICAL COMPOSITION

Fundamentally respiration is not a complex reaction. It is an oxidation-reduction reaction wherein one substance is oxidized, and another is reduced with the liberation of energy. In the case of most plants carbon is oxidized and oxygen reduced. The factors conditioning this oxidation-reduction are many and complex, however, and not well understood. It is affected by the formation of a suitable substrate, by the presence of enzymes, and by many and varied environmental conditions. SPOEHR (28) has pointed out that when carbohydrates are present in sufficient amounts, respiration is independent of carbohydrates, but proportional to the amino acids present, which, he claims, act in a catalytic manner. This does not seem to hold in the present case.

From tables IV and V the following relations between respiration and chemical composition can be drawn. Where respiration is most active there is (a) a greater insoluble nitrogen content, (b) a smaller soluble nitrogen content, (c) a smaller reducing sugar content, and (d) a greater phosphorus content, both soluble and insoluble. PALLADIN (23) states that, "When there is sufficient amounts of carbohydrates present the quantity of carbon dioxide exhaled by plants is directly proportional to the quantity of insoluble protein contained in them." In seedlings, the quantity of insoluble proteins is a measure of respiration.

Phosphorus, then, seems to influence respiration in two ways: (1) directly, by its association with carbohydrates to form a suitable substrate for the respiratory enzymes, and (2) indirectly, as it limits the amount of protein synthesis, and thereby the rate of growth.

Summary

1. Marquis spring wheat was grown to maturity in the greenhouse in a phosphorus-deficient nutrient solution. These plants yielded fewer and lighter seeds, which had a slightly higher protein content and a lower starch and phosphorus content than plants grown to maturity in a complete nutrient solution.

2. The respiration behavior of the above seeds was studied during germination on a nutrient solution lacking phosphorus and on a complete nutrient solution. The addition of phosphorus to the nutrient solution caused an increase in the production of carbon dioxide of about 10 per cent., in most cases, regardless of whether the seeds came from plants grown on the phosphorus-deficient nutrient solution or from plants grown on the complete nutrient solution.

3. Two species of wheat, including three varieties, were grown in nutrient solutions with or without phosphorus for a period of 7 days and the respiration measured during this period. At the end of the 7-day period other plants treated the same were analysed for reducing sugars, starch, soluble and insoluble nitrogen, and soluble and insoluble phosphorus. From this study the following conclusions are drawn:

a. The wheat plants grown on a complete nutrient solution respired approximately 6 per cent. more carbon dioxide than those plants grown on a nutrient solution lacking phosphorus.

b. Those plants grown on a complete nutrient solution as compared with those on a nutrient solution lacking phosphorus contained (1) more insoluble and less soluble nitrogen, (2) less reducing sugars, and (3) more insoluble and more soluble phosphorus.

c. No starch was found in the tops or roots of the wheat seedlings in any case. No sucrose was found.

d. In the present study respiration is not limited by the lack of available carbohydrates, but is limited by the lack of phosphorus.

e. Phosphorus, then, seems to influence respiration in two ways: (1) directly, by its association with carbohydrates to form a suitable substrate for the respiratory enzymes, and (2) indirectly, as it limits protein synthesis.

The writer wishes to acknowledge his appreciation for the encouragement and helpful suggestions offered by Professors C. A. SHULL and S. V. EATON. Thanks are also due the North Dakota Agricultural Experiment Station

and to the United States Department of Agriculture Experiment Station at Davis, California, for the seeds of Kubanka and Poso wheats.

UNIVERSITY OF CHICAGO

LITERATURE CITED

1. COCKEFAIR, E. A. The rôle of phosphorus in the metabolism of plants. Amer. Jour. Bot. **18**: 582–597. 1931.
2. Committee on Methods of Chemical Analysis. Determination of carbohydrates. Plant Physiol. **10**: 387–392. 1935.
3. EATON, SCOTT V. Influence of sulphur deficiency on the metabolism of the soy bean. Bot. Gaz. **97**: 68–100. 1935.
4. ECKERSON, S. H. Influence of phosphorus deficiency on the metabolism of tomato plants. Amer. Jour. Bot. **16**: 852. 1929.
5. ————. Influence of phosphorus deficiency on metabolism of the tomato (*Lycopersicum esculentum* Mill.). Contrib. Boyce Thompson Inst. **3**: 197–217. 1931.
6. GERICKE, W. F. The beneficial effect to wheat growth due to depletion of available phosphorus in the culture media. Science, n.s. **60**: 297–298. 1924.
7. GOTTSCHALK, A. Die Angriffspunkte der Co-zymase bei der Vergärung von Traubenzucker und Hexosediphosphorsäure. Zeitschr. physiol. Chem. **170**: 264–273. 1927.
8. HARDEN, A., and YOUNG, W. J. The alcoholic ferment of yeast-juice. Pt. III. The function of phosphates in the fermentation of glucose by yeast-juice. Proc. Roy. Soc. London B **80**: 299–311. 1908.
9. KRAUS, E. J., and KRAYBILL, H. R. Vegetation and reproduction with special reference to the tomato. Oregon Agr. Exp. Sta. Bull. 149. 1918.
10. KRAYBILL, H. R. Plant metabolism as an aid in determining fertilizer requirements. Ind. & Eng. Chem. **22**: 275–276. 1930.
11. LEVENE, P. A. Structure and significance of phosphatides. Physiol. Rev. **1**: 327–393. 1921.
12. LYON, C. J. The effect of phosphates on respiration. Jour. Gen. Physiol. **6**: 299–306. 1923.
13. ————. The rôle of phosphate in plant respiration. Amer. Jour. Bot. **14**: 274–284. 1927.
14. MAKINO, KATASHI. Über die Konstitution der Nucleinsäure. Zeitschr. physiol. Chem. **236**: 201–207. 1935.
15. MACGILLIVRAY, JOHN H. Effect of phosphorus on the composition of the tomato plant. Jour. Agr. Res. **34**: 97–127. 1927.
16. MELDRUM, NORMAN U. Cellular respiration. Methuen and Co. Ltd., London. 1934.

17. MITCHELL, JOHN W. A method of measuring respiration and carbon fixation of plants under controlled environmental conditions. Bot. Gaz. **97**: 376–387. 1935.
18. MCGINNIS, F. W. The effect of respiration upon the protein percentage of wheat, oats, and barley. Jour. Agr. Res. **24**: 1041–1048. 1923.
19. NEUBERG, C., and POLLAK, H. Über Kohlenhydratphosphorsäureester. 1. Über Saccharosephosphorsäure. Biochem. Zeitschr. **23**: 515–517. 1910.
20. NIEMANN, CARL, ROBERTS, R. H., and LINK, KARL PAUL. Isolation and determination of starch in plant tissue. Plant Physiol. **10**: 579–580. 1935.
21. NIGHTINGALE, G. T., ADDOMS, RUTH M., ROBBINS, W. R., and SCHERMERHORN, L. G. Effects of calcium deficiency on nitrate absorption and on metabolism in tomato. Plant Physiol. **6**: 605–630. 1931.
22. ————., SCHERMERHORN, L. G., and ROBBINS, W. R. Effects of sulphur deficiency on metabolism in tomato. Plant Physiol. **7**: 565–595. 1932.
23. PALLADIN, V. I. The dependence of the respiration of plants on their content of insoluble protein. Exp. Sta. Rec. **9**: 326. 1897.
24. PHILLIPS, THOMAS G. The determination of sugars in plant extracts. New Hampshire Agr. Exp. Sta. Sci. Contrib. no. 34. 1932.
25. PREGL, FRITZ. Quantitative organic microanalysis. P. Blakiston's Son and Co. Inc., Philadelphia.
26. PUCHER, G. W., LEAVENWORTH, C. S., and VICKERY, H. B. Determination of total nitrogen of plant extracts in presence of nitrates. Ind. & Eng. Chem. Anal. Ed. **2**: 191–193. 1930.
27. ROBISON, ROBERT. A new phosphoric ester produced by the action of yeast juice on hexose. Biochem. Jour. **16**: 809–824. 1922.
28. SPOEHR, H. A., and MCGEE, J. M. Studies in respiration and photosynthesis. Carnegie Inst. Washington. 1923.
29. ———— The oxidation of carbohydrates with air. Jour Amer. Chem. Soc. **46**: 1494–1503. 1924.
30. THOMAS, M. D. Precise automatic apparatus for continuous determination of carbon dioxide in air. Ind. & Eng. Chem. Anal. Ed. **5**: 193–198. 1933.
31. VON OHLEN, FLOYD W. A microchemical study of soy beans during germination. Amer. Jour. Bot. **18**: 30–49. 1931.
32. WOODWARD, J. Thoughts and experiments on vegetation. Phil. Trans. Roy. Soc. London **21**: 193–227. 1699.

TRANSPIRATION AS MODIFIED BY POTASSIUM[1]

ALBERT G. SNOW, JR.

(WITH FOUR FIGURES)

Introduction

Much accumulated evidence in regard to the possible functions of potassium in the metabolism of the green plant has been gained through a study of the behavior of plants grown in a medium lacking this element. In the numerous investigations dealing with the effect of potassium on transpiration, however, little work has been done in which the transpiration of plants grown without potassium has been investigated. In fact, most of the work has involved the interpretation of experiments in which the plants have been grown in single salt solutions, or solutions or soil cultures to which potassium salts have been added. Because of peculiarities brought about by the toxic effect of single salt solutions, or the unknown factors in dealing with soil as a medium, all of the evidence has not been in agreement. A fairly complete review of the pertinent literature may be found in the publications of several authors (**2, 3, 4, 7, 10, 11, 13, 14**). In view of the methods employed and the seemingly contradictory evidence gained in former experiments, it seemed desirable to reinvestigate this problem, using a different method of approach.

Materials and methods

All experimental work reported was carried on in a large greenhouse of the Yale Botanical Gardens during the spring and fall of 1934, and the winter of 1934–35. With the exception of the bean series, each experiment was performed at least twice and comparable results secured in each case.

Three species of plants were used, namely, *Helianthus annuus* L., *Phaseolus vulgaris* L., and *Nicotiana tabacum* L.[2] Shive's (**15**) R4C2 nutrient solution with a total osmotic pressure of 1.75 atmospheres was used as the control solution (*A*). Modifications of this were adapted for nutrient solutions lacking potassium. In one of the potassium-deficient solutions (*C*), no monovalent ion was added to take the place of potassium and the concentrations of the remaining salts were changed so as to maintain the same total molecular concentrations as those of the control solution. The second potassium-deficient solution (*D*) was similar to the control solution except

[1] Contribution from the Osborn Botanical Laboratory of Yale University.

[2] The sunflower seeds were furnished by Professor R. G. WIGGANS of Cornell University, and the Turkish variety of tobacco seed by Doctor M. F. MORGAN of the Connecticut Agricultural Experiment Station.

that sodium replaced the potassium in equivalent quantities. Table I indicates the salts and their partial molecular concentrations. Supplementary salts of iron, manganese, and boron were added. Their final concentrations were: iron 2 p.p.m., manganese 1.5 p.p.m., and boron 1.5 p.p.m.

TABLE I

PARTIAL MOLECULAR CONCENTRATIONS OF THREE TYPES OF SALT SOLUTIONS USED

SALTS	PARTIAL MOLECULAR CONCENTRATIONS		
	SOLUTIONS		
	A (CONTROL)	*C* (MINUS K)	*D* (Na SUBSTITUTED FOR K)
KH_2PO_4	0.0144	.....	.....
NaH_2PO_4	.....	.....	0.0144
$Ca(H_2PO_4)_2$	.....	0.0104	.....
$Ca(NO_3)_2$	0.0052	0.0026	0.0052
$Mg(NO_3)_2$	.....	0.0024	.....
$MgSO_4$	0.0200	0.0200	0.0200

The sunflower and bean seeds were planted in thoroughly washed white sand and watered with tap water until the first leaves were beginning to show. They were then watered with dilute control nutrient solution until the seedlings were transferred to the glass jars. The tobacco seeds were planted in a seed flat, in soil that had been sterilized at 135° C. for 2 hours.

The experimental plants were selected from a large group of seedlings for uniformity of size and apparent vigor of tops and roots. In each experiment, 10 plants were reserved for each solution used. Before transferring the seedlings to the culture solutions, the roots were thoroughly washed in tap water to remove any adhering sand or soil particles, and a strip of non-absorbent cotton wrapped around the stem. Each plant was then inserted in a 250-cc. glass jar which was wrapped in light brown paper, and contained solution *A*. After several weeks the plants were placed in liter jars, the roots being carefully drawn through a large cork, and another piece of cotton placed around the stem to support the plant.

In all the experiments the solutions were changed every 3½ days, and the jars thoroughly rinsed with tap water each time. In cases where they were placed into either solution *C* or *D*, the roots, as well as the jars, were rinsed with distilled water.

Extra light was provided throughout each experiment by two 500-watt Mazda bulbs equipped with 18-inch reflectors and suspended 25 inches above the plants. By use of an automatic time switch the electric lights were turned on at dusk and left on for 6 hours.

The water loss was determined by weighing each culture jar at stated intervals, the loss in weight being attributed to transpiration. In most cases the amount of water transpired was replaced with distilled water to maintain, as nearly as possible, a constant level of solution in the jar. Blueprints were made of all the leaves at the time of harvesting, and later the areas were determined with a planimeter.

The water loss is expressed in grams per square decimeter of leaf area (one side). All data were examined statistically according to STUDENT's method of determining the significance of difference of means of small samples (5).

In the graphical representation of results, the average water loss for each group of plants, during an interval, in grams per square decimeter of leaf area, is plotted. Below these values, a block diagram represents the differences occurring between the average transpiration rate of the plants in the potassium-deficient solution and those in the complete nutrient solution. A subscript 'S' under a bar indicates a significant difference between the means of the two series.

Experimentation

EXPERIMENT I

A comparison was made in experiment I between the transpiratory behavior of tobacco and sunflower plants in various nutrient solutions, under the same environmental conditions. This was done to secure a check on the trends in transpiration exhibited by former series of sunflower and tobacco plants grown at different times, but in the same nutrient solutions.

Three separate experiments, with sunflower plants, were performed using solutions *A*, *C*, and *D*. Two groups of tobacco plants were grown at the same time in solutions *A* and *C*. On March 19, transpiration measurements were begun. The sunflower plants were 4 weeks old, and the tobacco plants 9 weeks old. On March 23, one group of sunflower plants and one group of tobacco plants were placed in solution *C*, another group of sunflower plants was placed in solution *D*, and the two remaining groups of sunflower and tobacco plants were retained in solution *A* as the controls. All of the plants were harvested on March 30, and the green and dry weight, and leaf areas secured.

When the sunflower plants were harvested, the plants growing in the solutions lacking potassium were beginning to show signs of potassium starvation, as was seen in a yellowing of the lower leaves and inhibited marginal development. The tobacco plants in the solution lacking potassium did not show such marked signs of potassium deficiency, although the lower leaves were just beginning to show yellow spotting.

Figure 1 graphically represents the transpiratory behavior of the sunflower plants, and figure 2 that of the tobacco plants.

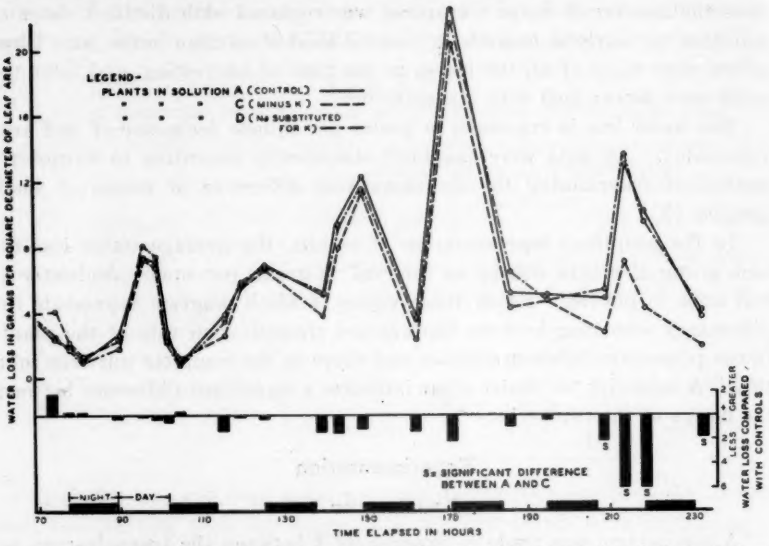

FIG. 1. Average transpiration rates of sunflower plants grown in solutions *A* (control), *C* (minus K), and *D* (Na substituted for K).

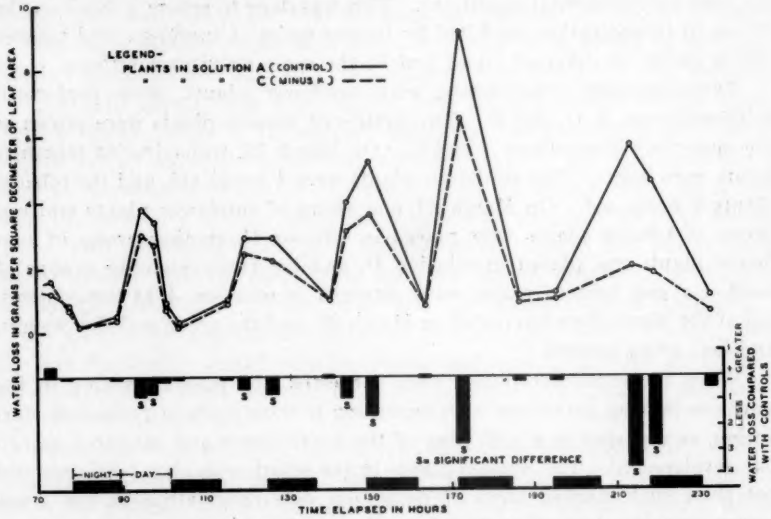

FIG. 2. Average transpiration rates of tobacco plants grown in solutions *A* (control), and *C* (minus K).

In general, the average transpiration rates of each group of tobacco plants, and each group of sunflower plants, were essentially similar for the first 70 hours, or up to the time the different groups were placed in their respective test solutions. At varying times after this, however, significant differences in transpiration rates occurred.

Examination of figure 1 shows that, for the sunflower plants, no differences in transpiration rates between the three groups were secured until the last 45 hours the plants were in the test solutions. The average transpiration rates of the plants growing in solution *D* (sodium substituted for potassium), compared with the average transpiration rates of the plants growing in solution *A* (control), exhibited no significant differences during the 162 hours in the test solution. On the other hand, the average transpiration rates of sunflower plants grown in solution *C* (lacking potassium) exhibited a different behavior. Similar to the sunflower plants in solution *D*, the transpiratory behavior of the sunflower plants in solution *C* closely paralleled that of the control plants for the first 125 hours in the test solutions. From this time to the end of the experiment, or during the next 45 hours, however, the average transpiration rate of the plants in solution *C* progressively became less than the average transpiration rate of the control plants. This inhibiting influence, toward the latter part of the experiment, is characteristic of plants grown in solution *C* in all the experiments conducted during this investigation. In addition, greater differences in transpiration rates occurred during the daylight intervals than during the night intervals. This is especially noticeable in the tobacco plants as illustrated in figure 2.

Another comparison may be made between tobacco and sunflower plants. From the data of this experiment, as illustrated in figures 1 and 2, it is seen that the sunflower plant inherently uses more water than the tobacco plant. The magnitude of the water loss per square decimeter of leaf area in the tobacco plant (for each 8-hour period) rarely exceeded 8 cc., being about 5 cc. most of the time. On the other hand, the water loss from the sunflower plants reached a maximum of nearly three times that of the tobacco plants, losing as much as 23 cc. of water per plant in an 8-hour period, and averaging about 10 cc.

The transpiratory behavior of the tobacco plants may be contrasted with that of the sunflower plants in another way. The decrease in transpiration exhibited by the tobacco plants so soon after being placed in the solution lacking potassium, in comparison with sunflower plants, seems to indicate that tobacco plants have a greater sensitivity to various nutrient solutions, in relation to transpiration, than do the sunflower plants. In the case of tobacco, the transpiration rate decreased, in comparison with the

controls, 20 hours after the plants were placed in the solution lacking potassium, while sunflower plants did not show significant differences until 125 hours after being placed in the solution lacking potassium. This decrease in the average transpiration rate was also more evident during the daylight intervals, when the environmental conditions were conducive to high transpiration rates, than they were during the night intervals, when no differences in transpiration rates were noticed until the latter part of the experiment.

Experiment II

The transpiratory behavior of two groups of tobacco plants was studied in experiment II, and the same procedure was followed as in the preceding experiment. The plants used were 2 weeks younger than in experiment I, and were grown in solution *D*. This solution had sodium substituted for potassium, while solution *C*, as used in experiment I, contained neither sodium nor potassium. Although the plants were not grown at the same time as those in the former experiment, the environmental conditions were essentially similar. The results are graphically depicted in figure 3.

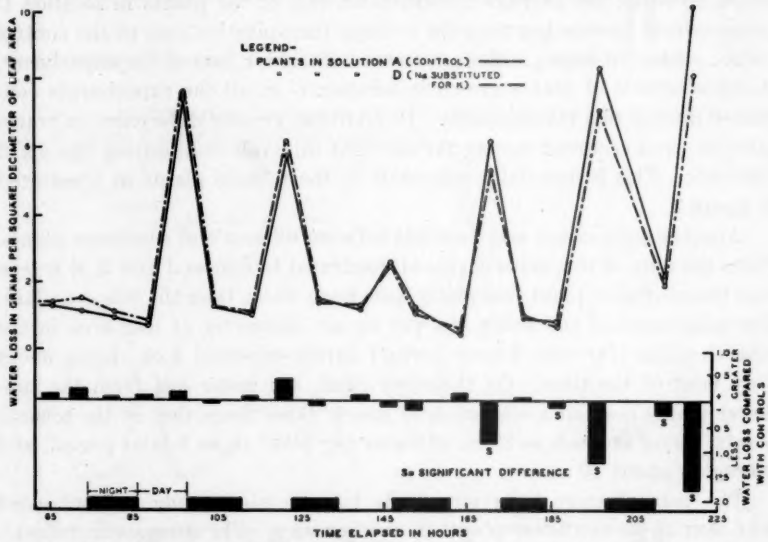

Fig. 3. Average transpiration rates of tobacco plants grown in solutions *A* (control), and *D* (Na substituted for K).

In this experiment, the transpiration rate of the tobacco plant again appeared to be affected by variations in the nutrient solutions to a greater degree than that of the sunflower plant. In the sunflower plants of experi-

ment I, no significant differences in transpiration rates occurred between plants grown in solutions *A* and *D* for the duration of the experiment. In experiment II significant differences in transpiration rates occurred between the tobacco plants grown in solutions *A*, and *D*. A significant difference in transpiration rate appeared after the plants were in the test solution 96 hours. The rate of water loss of the plants in solution *D* was less than that of the controls. This decrease in rate of transpiration became progressively greater (during the daylight intervals) up to the time the plants were harvested.

The greater differences in transpiration rates, observed in the latter part of the experiment, were also associated with a greater quantity of water loss. In other words, when a greater need for water occurred, either the plants deprived of potassium were less able to supply this water, or the water was more tenaciously held than in the plants supplied with potassium. This occurred only during the daylight intervals.

Experiment III

In order to determine whether or not the same general trends were exhibited by another species of plant, the common bean was chosen for experiment III. The same general procedure was followed as in the previous experiments. A further departure was instituted in this series: Two-thirds of the plants in each group were returned to the complete nutrient solution (*A*), after being in the test solutions one week, and allowed to grow another 7 days. This experiment provided data on the rate of transpiration which was similar to that in experiment II, and, in addition, showed the time required and to what degree bean plants recovered from potassium starvation in regard to their rate of transpiration.

The transpiration rates of these plants are given in figure 4. In this experiment the data on transpiration were calculated on the basis of the dry weight of the leaves. While these data, therefore, are not strictly comparable to those of former experiments, the results show the same general trends of the effects of the test nutrient solutions on transpiration. This follows from a comparison of the results secured in former experiments by basing water loss on both leaf area and dry weight.

It was found that the difference in the rate of transpiration of the plants in solution *D*, as compared with the controls, was not significant in any instance. On the other hand, significant differences occurred in the transpiration rates of the plants in solution *C* as compared with the control plants in solution *A*.

After placing the plants in the test nutrient solutions, the transpiration rates of the group of plants, grown in solution *C*, and the controls, were

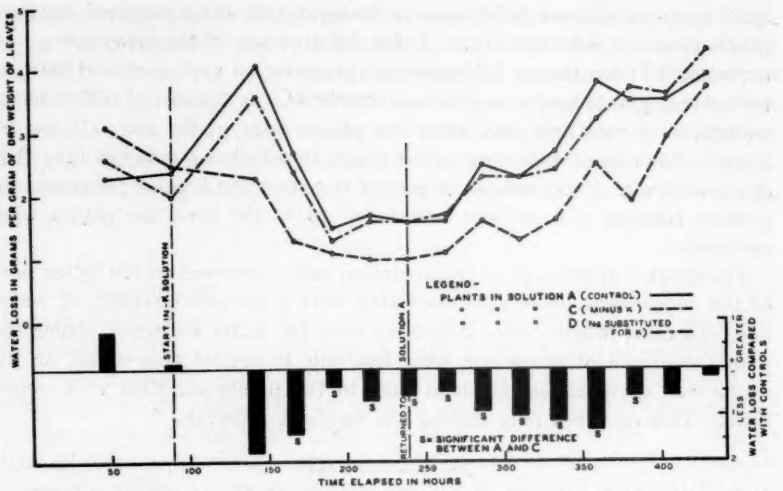
WATER LOSS IN GRAMS PER GRAM OF DRY WEIGHT OF LEAVES
START IN K SOLUTION
SOLUTION A RETURNED TO
LEGEND-
PLANTS IN SOLUTION A (CONTROL)
C (MINUS K)
D (Na SUBSTITUTED FOR K)
S= SIGNIFICANT DIFFERENCE BETWEEN A AND C
GREATER
LESS
WATER LOSS COMPARED WITH CONTROLS
TIME ELAPSED IN HOURS

FIG. 4. Average transpiration rates of bean plants grown in solutions *A* (control), *C* (minus K), and *D* (Na substituted for K).

essentially similar for a period of 54 hours. From this time on, until the plants in solution *C* were returned to the complete nutrient solution (*A*), the transpiration rate of the plants in the potassium-deficient solution was significantly less than the controls. When the plants in solution *C* were again placed in the complete nutrient solution, the transpiration rate of these plants progressively decreased, as compared with the controls, for a further period of 122 hours. The transpiration rates then approached more nearly those of the controls during the next 20 hours. Finally, no significant difference in the transpiration rates of either group of plants was noted for the remaining 50 hours of the experiment.

The results of the experiment seem to indicate that, in the bean plant, a lack of potassium in the nutrient medium inhibits transpiration to a marked degree after an interval of several days. On being returned to the complete nutrient solution, the inhibiting influence seems to remain for a time, and, after a further interval of several days, the transpiratory behavior returns to normal.

Discussion

Although sunflower and tobacco plants have been studied by many workers in their relation to transpiration, very little work has been done with these species with reference to the influence which potassium plays in modifying their transpiration rates. Numerous investigations have been

conducted in which plants were grown in single salt solutions, but, with the exception of an observation by HARTT (9), few references dealing with studies of the influence of potassium on transpiration have been found in which the plants were grown in a nutrient solution with only potassium omitted, or with sodium substituted for potassium.

It was found in these experiments that when normal healthy plants were placed in a solution lacking potassium, after an interval of time the transpiration rates of these plants became progressively less than those of the controls. HARTT (9), working with sugar cane growing in sand cultures in which the nutrient supply contained no potassium, made the observation that the transpiration rate of the potassium-deficient plants was less than that of the control plants. The results of the writer's work on tobacco, bean, and sunflower plants substantiate the observation made by Miss HARTT, and give additional information as to the condition of the plants at the time, and their subsequent behavior. In fact, the time element is an important consideration, because after a more or less definite interval, depending on the species, upon placing the plant in the test nutrient solution, transpiration is inhibited.

It was further observed that, after the progressive decrease in transpiration had commenced, these differences were greater during the daylight intervals than during the night intervals. Several reasons may account for this. First, it was found that when these greater differences in transpiration rates occurred, the magnitude of this water loss was the greatest. Secondly, the temperature was usually high and the relative humidity low during these intervals. It may be that greater differences occurred because of the inability of the plants, grown in the potassium-deficient solution, to supply a sufficient amount of water for transpiration as a result of the increased demand. This inability may have been caused by the decreased efficiency of the roots to absorb water. After being in the potassium-deficient solution several days, the roots of these plants appeared brown, and were not elongating as rapidly as the roots of the control plants. On the other hand, the water-holding mechanism of the leaves may have been affected by growing the plants in the potassium-deficient solution. After a certain amount of dehydration of the leaf cells had occurred, the remaining water may have been more tenaciously held, thereby lessening the transpiration rate. This latter possibility seems more probable because, when this decrease in transpiration rate occurred, the plants did not wilt. If the absorptive power of the roots had diminished, one would be led to believe that, after a certain amount of transpiration, the leaves would wilt because of a lack of supply of water from the roots. This was not the case. The greater decrease in water loss during these intervals, however, may have been due to a com-

bination of both of these conditions, and, in addition, there may have been other factors influencing transpiration.

It is possible that changes in the stomatal cycle occurred when the plants were deprived of potassium, thereby bringing about a change in transpiration rate. If the lack of potassium caused an accumulation of sugars or starch, a change in the acidity of the cell sap, or a change in the osmotic concentration of the cell sap, the opening or closing of the stomata may have been inhibited. No observation was made on these phases of the problem in the present experiments.

By omitting potassium from a nutrient solution, BARTHOLOMEW, *et al* (**1**), HAAS and REED (**6**), and others, found that the absorption of calcium, nitrogen, and phosphorus was increased. According to UDOLSKAYA (**17**), the action of phosphorus on plants consists largely in "increasing the water-retaining capacity of the plasma." If this is the case, the decrease in transpiration noted in these experiments may be due, in part, to the increased water-holding capacity of the leaf because of the increased amount of phosphorus taken in by the plant. In this connection, SHREVE (**16**) concluded that variations in transpiring power are due to changes in the water-holding capacity of internal tissue. SHREVE believes that a decrease in transpiration is brought about by an increased water-holding capacity of the cell colloids, which act indirectly by causing the closing of the stomata.

If translocation is inhibited by a lack of potassium in the nutrient solution, as some workers postulate, there would possibly be an accumulation of proteins in the leaf. If this occurs, according to MOTHES (**12**), the leaves rich in proteins lose less water than the leaves that are low in protein, in the same amount of time, owing to their higher hydrophilic colloid content. This might explain, in part, the trends noted in these experiments, except that nitrogen metabolism is also inhibited by a lack of potassium.

It appears from the present experiments that sodium may, to some extent, take the place of potassium in relation to transpiration. For instance, in the experiment with bean plants, the transpiration rate of the plants grown in the test nutrient solution, in which sodium was substituted for potassium, did not vary significantly from that of the control plants. The plants grown in a potassium-deficient solution, in which there was no sodium substituted for potassium, on the other hand, exhibited a decrease in transpiration rate after about four days. The same trends were evident in the tobacco and sunflower plants used in these experiments. HARTT (**9**) stated that sodium cannot take the place of potassium in the water relations of a plant. This conclusion is in agreement with the results of the writer's investigation, if one considers that Miss HARTT's observations were based on a long time experiment in which the plants used had grown continuously in the potassium-deficient solution. It is desired to emphasize that, for a comparatively short interval of

time, it seems that sodium can function in the place of potassium in relation to transpiration.

An examination of the pertinent literature seems to indicate that there is no single adequate explanation that will account for the differences in transpiration observed in this investigation. It may be reiterated that, when growing plants in a nutrient solution lacking potassium, one cannot ascribe to potassium all the plant responses that take place. The mere omission of potassium from the nutrient solution exerts a decided influence on the absorption of other elements, and these, in turn, may produce the observed effects. The relationship that exists between the chemical constituents in a nutrient solution and their effects on plant transpiration are little understood. Much work remains to be done on the correlation of the transpiratory responses of green plants with variations in nutrient solution.

Summary

1. When sunflower, tobacco, or bean plants are grown in a nutrient medium lacking potassium, the transpiration rate decreases after an interval of time.

2. The data of these experiments seem to indicate that the sunflower plant inherently uses more water than the tobacco plant.

3. The tobacco plant responds more quickly in its rate of transpiration, owing to a lack of potassium in the nutrient solution in which it is grown, than does the sunflower plant.

4. The influence on the rate of transpiration in plants grown in a nutrient solution lacking potassium is quicker than in plants grown in a nutrient solution in which sodium replaces the potassium.

5. The greatest decreases in transpiration rates of plants starved of potassium occurred during the periods of highest light intensity and temperature; that is, when environmental conditions normally increase transpiration.

6. A discussion of the possible explanations for the observed experimental facts is given.

The writer wishes to express his appreciation for the generous counsel and assistance received from Professor CARL G. DEUBER during the investigation, and for aid given in the preparation of the manuscript.

NORTHEASTERN FOREST EXPERIMENT STATION
NEW HAVEN, CONNECTICUT

LITERATURE CITED

1. BARTHOLOMEW, R. P., WATTS, V. M., and JANSSEN, G. Effect of variations in the nutrient media upon the nitrogen, phosphorus, and

potassium content of plants with special reference to the tomato. Arkansas Agr. Exp. Sta. Bull. 288. 1933.

2. Bouyoucos, G. J. Transpiration of wheat seedlings as affected by soils, by solutions of various densities, and by various chemical compounds. Jour. Amer. Soc. Agron. **3**: 130–191. 1911.
3. Burgerstein, A. Die Transpiration der Pflanzen. I. pp. 141–149. Jena. 1904.
4. ————. Die Transpiration der Pflanzen. II. pp. 95–118. Jena. 1920.
5. Fisher, R. A. Statistical methods for research workers. pp. 107–112. 3rd ed. London.
6. Haas, A. R. C., and Reed, H. S. The absorption of ions by citrus and walnut seedlings. Hilgardia **2**: 67–106. 1926.
7. Hansteen-Cranner, B. Über das Verhalten der Kulturpflanzen zu den Bodensalzen III. Jahrb. wiss. Bot. **53**: 536–602. 1914.
8. Harter, L. L. The influence of a mixture of soluble salts, principally sodium chloride, upon the leaf structure and transpiration of wheat, oats, and barley. U. S. Dept. Agr. Bur. of Plant Ind. Bull. 134. 1908.
9. Hartt, C. E. Some effects of potassium upon the growth of sugar cane and upon the absorption and migration of ash constituents. Plant Physiol. **9**: 399–452. 1934.
10. Kisser, J. Untersuchungen über den Einfluss der Nährsalze auf die Wasserabgabe, Wasseraufnahme, relative Spross- und Wurzelmasse und die Blattstruktur. I. Planta **3**: 562–577. 1922.
11. Meyer, B. S. Effects of mineral salts upon the transpiration and water requirements of the cotton plant. Amer. Jour. Bot. **18**: 79–93. 1931.
12. Mothes, K. Zur Kenntnis des N-Stoffwechsels höherer Pflanzen. Planta **12**: 686–731. 1931.
13. Reed, H. S. The effect certain chemical agents upon the transpiration and growth of wheat seedlings. Ann. Bot. **49**: 81–101. 1910.
14. Schmucker, Theodor. Über den Einfluss narkotischer Stoffe auf Transpiration und Wasserleitung. Jahrb. wiss. Bot. **68**: 771–800. 1928.
15. Shive, J. W. A study of physiological balance in nutrient media. Physiol. Res. **1**: 327–397. 1915.
16. Shreve, E. B. An analysis of the causes of variations in the transpiring power of cacti. Physiol. Res. **2**: 73–127. 1916.
17. Udolskaya, N. L. Study of the elements of mineral nutrition as factors in changing the drought resistance of plants. Compt. Rend. Acad. Sci. U.R.S.S. (N.S.) **2**: 45–46. 1934. (C. A. **28**: 5100. 1934.)

COMPARATIVE EFFECTS OF ALTERING LEAF TEMPERATURES AND AIR HUMIDITIES ON VAPOR PRESSURE GRADIENTS

OTIS F. CURTIS

(WITH TWO FIGURES)

In a recent paper the writer (**2**) presented curves showing comparisons between leaf and air temperatures under several environmental conditions. The data clearly indicated that in strong light, either direct sunlight or artificial light, leaves are likely to have temperatures from 2° to 10° C. or more above those of the air. In that paper brief mention is made concerning the importance of a consideration of leaf temperature when interpreting experiments dealing with the effects of various factors upon transpiration. A failure to recognize the fact that leaf temperature may be very different from that of the air, and thus have a marked effect on transpiration, has often led to misinterpretation or questionable interpretation of the influence of various environmental and leaf factors on transpiration.

It is often assumed that divergences of leaf temperature from air temperature are of no great significance in affecting transpiration. Differences of only a few degrees, however, may have a marked effect on transpiration, and when leaf temperatures are from 5° to 10° C. above those of the air the possible effects on transpiration may be very great and even in excess of any possible changes in air humidity. Assuming no change in the leaf area or in the stomatal condition, as well as no change in the kind of gas or the total pressure of gas (air) around the leaf, the rate of transpiration will be determined by the vapor pressure gradient.

When the intercellular spaces are in vapor pressure equilibrium with the living cells, the intercellular relative humidity probably rarely falls below 97 per cent., because this would be in equilibrium with cells with a turgor deficit or suction tension of 42 atmospheres (**4**). If, on the other hand, the cell wall or protoplasmic membrane has a low permeability to water, and loss of water vapor to the atmosphere is rapid, the relative humidity of the intercellular spaces may fall considerably below 100 per cent. For the sake of simplicity, however, it is assumed that the intercellular relative humidity remains at 100 per cent. The rate of air movement over the leaf will of course affect the relative humidity close to the leaf and therefore the gradient; but it is assumed further that for any comparisons here made the air movement does not vary. Under these assumptions the vapor pressure gradient may be altered by changing the relative humidity in the external atmosphere, when keeping the temperature constant, or it may be altered by changing either the temperature of the leaf or that of the atmosphere about the leaf.

A specific example of such a condition follows. If the leaf and air temperatures are both 20° C., the leaf relative humidity 100 per cent., and the air relative humidity 60 per cent., the vapor pressure in the leaf will be 17.36 mm. mercury, that in the atmosphere will be 10.42 mm., and the gradient will be determined by the difference in vapor pressure, which is 6.94 mm. The actual gradient will be dependent further upon the number and size of the stomata, the size, shape, and orientation of the leaf, the nearness to other leaves and rate of air flow; but, as stated above, these factors are assumed to be invariable. If now the temperature of the leaf is raised 1° C. above that of the air, or to 21° C., assuming 100 per cent. relative humidity within the leaf (and the relative humidity is likely to remain close to this figure because of the large cell surface exposed in the intercellular spaces), the vapor pressure within the leaf will rise to 18.47 mm., thus increasing the vapor pressure difference to 8.05 mm., and steepening the gradient. The rise in vapor pressure within the leaf owing to this 1° C. rise in temperature is 1.11 mm., (18.47–17.36 mm.) which is 6.4 per cent. of the vapor pressure at 20° C. To bring about the same difference in vapor pressure by lowering the external relative humidity, it would be necessary to lower the vapor pressure of the atmosphere by an amount equivalent to 6.4 per cent. of the vapor pressure at saturation. This can be brought about by lowering the relative humidity of the atmosphere by 6.4 per cent., that is from 60 to 53.6 per cent. Beginning at 20° C., therefore, a rise in leaf temperature of 1° C. above that of the air will have an effect on the vapor pressure gradient equivalent to lowering the external humidity 6.4 per cent. The actual initial increase in vapor pressure is independent of the original atmospheric humidity, because it is expressed as a percentage of the pressure at saturation. That is, raising the leaf temperature 1° C., beginning at 20° C., will have the same effects on vapor pressure gradients as lowering the external humidity 6.4 per cent., from 100 to 93.6 per cent., 75 to 68.6 per cent., or 46.4 to 40 per cent. The relative effect on total gradient and therefore on transpiration, whether brought about by rise in leaf temperature or lowering of external humidity, of course would be much greater the higher the original humidity. For example, with leaf and air at the same temperature, 20° C., and the air at 100 per cent. relative humidity, there would be no transpiration (no net loss), and raising the leaf temperature 1° C. or lowering the external humidity 6.4 per cent. would allow for appreciable transpiration and the increase would be infinite; if the original atmospheric humidity were 93.6 per cent. raising the leaf temperature 1° C. or lowering the atmospheric humidity 6.4 per cent., that is to 87.2 per cent., would double the gradient and therefore tend to double the rate of transpiration; whereas, if the original external humidity were 6.4 per cent., raising the temperature of the leaf 1° C. or lowering the external humidity 6.4 to 0 per cent. would have a relatively slight effect on the rate of transpiration.

In a similar way, a rise of leaf temperature to 5° C. above that of the air, that is from 20° to 25° C., will increase the vapor pressure gradient by 6.16 mm. (23.52–17.36 mm.), which would be equivalent to lowering the external humidity by 35.5 per cent. Raising the leaf temperature 10° C., from 20 ° to 30° C., would increase the vapor pressure in the leaf to 31.5 mm., that is, by 14.15 mm., and this would have an effect on the vapor pressure gradient equivalent to lowering the atmospheric humidity by 81.5 per cent. From this it is evident that if the leaf and air temperatures are the same, and at 20° C. or lower, and the air humidity is 80 per cent., raising the leaf temperature 10° C. above the air temperature would increase transpiration to a

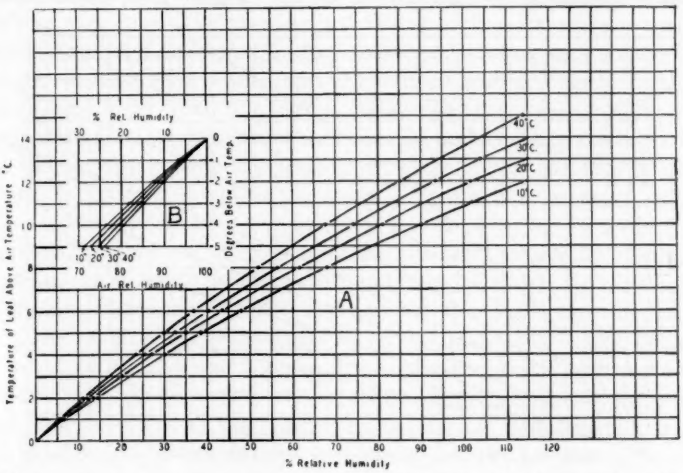

FIG. 1. *A*, effect on vapor pressure gradient of raising leaf temperature above air temperature in terms of lowering the relative humidity. Leaf temperatures at start are at the four air temperatures 10°, 20°, 30°, and 40° C. Intercellular atmosphere of leaf remains saturated. *B*, effect on vapor pressure gradient of lowering leaf temperature below air temperature in terms of increasing the humidity of the air.

greater extent than would lowering the external humidity from 80 to 0 per cent.

Curves showing the equivalent effects of raising the leaf temperature in terms of lowering the relative humidity, for beginning temperatures of 10°, 20°, 30°, and 40° C., are presented in figure 1, *A*. The data for these curves were calculated, as indicated above, from the vapor pressure data given in the *Handbook of Chemistry and Physics*. The equivalent lowering of relative humidity will, of course, be greater the lower the original temperature, because the change in vapor pressure is expressed as a percentage of the original vapor pressure. From the curves it is obvious that the vapor pres-

sure gradient, and therefore transpiration, may, in many cases, be increased to a greater extent by raising the leaf temperature than can possibly be brought about by lowering the external humidity, because raising the leaf temperature may steepen the gradient more than would result on lowering the external humidity to 0 per cent. This is especially true at low air temperatures or low air humidities.

If the leaf temperature becomes lower than the air temperature, and this may frequently occur on clear nights as a result of radiation to space, or under laboratory conditions by radiation to cold objects (**2**), then the effect on the vapor pressure gradient and transpiration will be comparable to that of increasing the humidity of the air. Figure 1, *B*, shows the effect

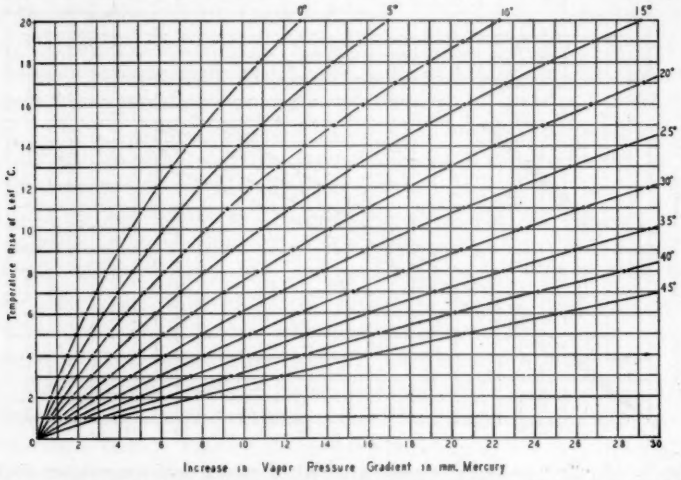

FIG. 2. Curves showing increases in vapor pressure gradients, expressed as millimeters of mercury, resulting from rises in leaf temperatures over air temperatures at the assumed initial air temperatures of 0° to 45° C., with 5° intervals. Intercellular spaces are assumed to remain saturated.

of lowering the leaf temperature below air temperature in terms of an equivalent effect of increasing the humidity of the air, as shown in the percentages across the top of the graph. These same curves also indicate the lowering of temperature that is necessary to bring the leaf to the dew point at the relative humidities of the air shown in the percentages across the bottom of the graph. As briefly discussed in a previous paper (**2**), this approach to saturation at the leaf surface probably plays an important rôle in raising the humidity at the surface of the leaf to a point where it becomes possible for fungous spores and bacteria to obtain enough water for germination or growth (**4**).

Instead of expressing the increased vapor pressure gradient resulting from a rise in leaf temperature in terms of an equivalent lowering of the external humidity, as presented in figure 1, *A*, the steepened gradient may be directly expressed as an increased pressure gradient in terms of millimeters of mercury. Such data are presented in figure 2 for initial temperatures ranging between 0° and 45° C. at 5° intervals. From this figure it is evident that the increase in actual vapor pressure gradient is higher, the higher the original temperature.

This fact, that with a given rise in temperature the gradient rises more steeply the higher the original temperature, would easily account for the findings of BIALOGLOWSKI (**1**) that transpiration was higher at higher temperatures even when the relative humidities of the atmosphere were adjusted to give the same vapor pressure deficits. Working with similar citrus plants in the same control chambers, the writer (**2**) found the leaf temperatures in light to be from 3° to 10° C. above the air. Assuming the atmosphere within the leaf to be saturated and the leaf temperature to be 5° C. above the air, the vapor pressure gradient at an air temperature of 30° C., as shown in figure 2, would be increased by 10.3 mm. of mercury, while at an air temperature of 10° C. it would be increased by only 3.5 mm. That is, instead of a gradient of 2.5 mm. at the two temperatures as assumed by BIALOGLOWSKI, it would be 12.8 at 30° and only 6.0 at 10° C. At the intermediate air temperatures of 25°, 20°, and 15° C. a leaf temperature excess of 5° C. would steepen the gradients by 8.0, 6.1 and 4.7 mm., and the gradients would be 10.5, 8.6 and 7.2 instead of the assumed 2.5. From a consideration of these gradients it is obvious that although BIALOGLOWSKI kept the vapor pressure deficit of the atmosphere constant at different air temperatures, the difference in vapor pressure between leaf and air, that is the gradient, was much steeper when the leaf was warmer than the air, and therefore transpiration was greater at higher temperatures. Only when the leaf temperature is the same as the air temperature will the same vapor pressure deficit of the atmosphere give the same gradient and therefore equal transpiration at different air temperatures.

Although the data for these curves have been calculated on the assumption that the air temperature remains constant while the leaf temperature alone changes, they can also be taken to indicate a possible maximum increase in gradient which might result if both leaf and air temperatures rise equally and simultaneously. This maximum would obtain only if the absolute humidity of the atmosphere, that is, the amount of water vapor, remains constant close to the leaf. This would be possible under natural conditions only when air movement is sufficiently increased to carry away the increased moisture coming from the leaf. On such an assumption the relative humidity of the atmosphere will, of course, decrease, but the vapor pressure may in-

crease slightly (at constant volume) or remain constant (at constant pressure). If the air movement is not increased as its temperature rises, transpiration will increase less when both leaf and air temperatures rise than when air temperature remains constant and leaf temperature alone rises. This is due to the fact that the back pressure of the vapor, which has come from the leaf, will be greater when the air temperature is high than when it is low. For example, with a leaf at 20° C. and the air at 20° C., the leaf at saturation will have an internal vapor pressure of 17.36 mm. The air at 60 per cent. relative humidity will have a vapor pressure of 10.42 mm. and the difference will be 6.94 mm. If the leaf temperature rises to 30° C., at saturation its internal vapor pressure would rise to 31.51 mm. This would hasten loss of water from the leaf and increase the amount of water vapor in the atmosphere close to the leaf. If the air remains at 20° C. the maximum vapor pressure here at saturation could be only 17.36 mm., and there would still be a rather steep gradient from leaf to air and a rapid transpiration. If, on the other hand, the air temperature also rises to 30° C., the vapor pressure close to the surface of the leaf, as it approaches saturation (which is likely to take place especially in rather quiet air), would approach a maximum vapor pressure of 31.51 mm. and the gradient would be much less than when the air temperature remains low. From this it is obvious that, unless accompanied by rapid air movement, a rise in leaf temperature above air temperature will be much more effective in hastening transpiration than would a simultaneous rise of both leaf and air temperature. One of the most widespread misconceptions concerning the influence of atmospheric conditions on transpiration is that a high air temperature favors transpiration because with a rise in air temperature its relative humidity decreases, its water holding capacity increases, and its vapor pressure deficit increases. Actually a rise in air temperature will increase transpiration only if it also raises the leaf temperature; and the increased transpiration is due solely to this rise of leaf temperature. In fact, transpiration would increase to a greater extent if the leaf temperature alone could be raised or if the leaf temperature remained constant and the air temperature could be lowered.

An appreciation of the influence on vapor pressure gradients of temperature differences and equivalent humidity differences, as here presented, may also be useful in a consideration of the recent proposal of PEIRCE (3) that, especially in arid regions, water may be moving in the vessels of trees as a vapor and not as a continuous column of liquid water. The proposal is that the loss of water from the cells of a leaf by transpiration increases the turgor deficit (suction tension) of these cells and lowers their vapor pressure. This lowered vapor pressure is assumed to result in a distillation of water to the leaves from the roots where the osmotic concentration is lower and therefore the vapor pressure supposedly higher. Of course, the move-

ment of water vapor in an atmosphere of air or other gas at nearly normal pressure would be extremely slow; but if the vessels contain only water vapor, the rate of diffusion would be immensely faster and the proposed hypothesis seems more nearly possible, although one may well wonder why such a partial vacuum containing only water vapor would not fairly quickly become filled with other gases diffusing through the wet walls. A still greater and, it seems, insurmountable difficulty is that, if the receiving cells of the leaf or twig had a temperature only 1° C. above the water surface from which the vapor is supposedly coming, the vapor pressure gradient would be reversed and the water would distill back into the roots. For example, if the leaf were at 21° C. and the water surface in the root at 20° C., the vapor pressure of pure water in the leaf would be 6.4 per cent higher than that in the root. The leaf would require an osmotic turgor deficit (suction tension) equivalent to 93 atmospheres to bring its vapor pressure down to a point equal to that of the root, while to lower it to a vapor pressure below that of the root, so as to bring about a diffusion gradient towards the leaf, a still greater turgor deficit would be necessary.

From these considerations, in which a temperature excess in the leaf of only 1° C. is shown to develop a vapor pressure gradient from the leaf to the water supplying tissue greater than can be offset by any reasonable osmotic suction, it is evident that water cannot be moving through the conducting tissues as a vapor, because the receiving tissues frequently have temperatures not merely 1° in excess of the supplying tissues but often 5° to 10° or more in excess. Under such conditions water, if it moved as a vapor, would not merely cease moving to the leaf but would quickly distill from the leaf to the supplying tissue. It would make no difference whether one assumed movement as a vapor over the entire distance from root to leaf or over any intermediate distance, as from root to twig or branch, or from the base of the trunk to the base of the branch; a temperature excess as small as 1° C. at the upper surface would cause a distillation downward faster than any osmotic lowering of the vapor pressure could cause a distillation upward.

Even a local lowering of the temperature anywhere along the stem would lower the vapor pressure at that point so that it could not move as a vapor from that region to another region at a higher temperature. PEIRCE obtained definite wilting only when the stem was frozen, which obviously would stop flow of liquid water. Although the leaves, twigs, or branches may frequently be cooler than the supplying tissues during the night, for the greater part of the day the upper receiving tissues in direct sunlight undoubtedly have temperatures much in excess of the supplying tissues and therefore could not receive water in the form of vapor. It would seem, therefore, that

water cannot move through the conducting tissues as a vapor in amounts that do move during rapid transpiration but must move as a liquid.

Although no new experimental data are here presented, the widespread failure to recognize the importance of the effect of leaf temperature on transpiration, and therefore the rather frequent partial misinterpretation of experiments involving transpiration, made it seem worth while to present these curves. Furthermore, although many have recognized the importance of leaf temperatures in transpiration, the writer has seen no attempt to express the relationships in curves which are as easily interpreted as those shown in figures 1 and 2 of this paper.

Summary

1. A factor of major importance influencing transpiration from leaves is the vapor pressure gradient from leaf to air. That this gradient is partly determined by the relative humidity of the air is universally recognized, but the fact that this gradient is also greatly affected by temperature differences between the leaf and air is often overlooked or underestimated.

2. Data are presented showing that a rise in leaf temperature of 5° C. above the air temperature, which is frequently exceeded by leaves in nature, may increase the vapor pressure gradient by amounts equivalent to those resulting from lowering the external relative humidity by from 30 to nearly 38 per cent., depending on the air temperature. A rise to 10° C. above the air, which is not uncommon, would be equivalent to lowering the humidity by 67 to 90 per cent., or to increasing the vapor pressure gradient by 8 to 30 mm. of mercury.

3. Curves are presented showing the effects in terms of the equivalent lowering of the relative humidity on the vapor pressure gradient resulting from raising the leaf temperature. Other curves give the effects of rises in leaf temperature on vapor pressure gradients in terms of millimeters of mercury. On the assumption of no change in amount of water vapor in the atmosphere, these same curves can be used to predict the maximum possible effects of an equal rise of both leaf and air temperatures on the vapor pressure gradient between leaf and air. A rise in air temperature corresponding to that of the leaf will ordinarily tend to reduce transpiration to a rate less than would take place if the air temperature remains low and the leaf temperature alone rises. The widespread concept that a rise in air temperature increases transpiration because the relative humidity is thereby lowered, or that the air is less saturated, or that it has a higher vapor pressure deficit, is a false concept, for the increased transpiration is due solely to a rise in leaf temperature.

CORNELL UNIVERSITY
ITHACA, NEW YORK

LITERATURE CITED

1. BIALOGLOWSKI, J. Effect of humidity on transpiration of rooted lemon cuttings under controlled conditions. Proc. Amer. Soc. Hort. Sci. **33**: 166–169. 1935.
2. CURTIS, OTIS F. Leaf temperatures and the cooling of leaves by radiation. Plant Physiol. **11**: 343–364. 1936.
3. PEIRCE, GEORGE J. Are living cells involved in the ascent of sap? Amer. Jour. Bot. **23**: 159–162. 1935.
4. SHAW, LUTHER. Intercellular humidity in relation to fire-blight susceptibility in apple and pear. Cornell Univ. Agr. Exp. Sta. Memoir 181. 1935.

CHEMICAL NATURE OF THE OXYGEN-TRANSFERRING FERMENT OF RESPIRATION IN PLANTS

WALTER KEMPNER

(WITH THREE FIGURES)

Introduction

CHEMICAL NATURE OF OXIDATION FERMENTS IN ANIMAL CELLS AND BACTERIA

The effect of carbon monoxide on the respiration of animal cells and aerobic bacteria (**10**), and on anaerobic butyric acid fermentation (**6**), has shown that the ferments which act as catalysts in these energy-yielding cellular processes are compounds of heavy metals: for carbon monoxide reacts at low temperatures only with heavy metals, and respiration as well as anaerobic butyric acid fermentation is stopped at low temperatures by carbon monoxide (**6, 10**). It has been further shown that these catalysts of respiration and of the anaerobic butyric acid fermentation are compounds of iron: for, of all the combinations of carbon monoxide with heavy metals only those with iron are dissociated by visible light, and the cell which in the presence of carbon monoxide stops respiring or forming butyric acid, recommences its metabolic reactions when irradiated by light (**7, 10, 11**).

Two iron ferments, the oxygen-transferring ferment of respiration and the anaerobic-splitting ferment, are apparently in too small a concentration and are too unstable to be isolated from the living cells by the usual methods of analytical chemistry. By combining them with carbon monoxide and by measuring the various degrees of dissociation of the ferment-CO combinations (by the increase of the respiration or of the anaerobic-splitting metabolism when irradiated by different wave lengths of visible light) an indirect method was found for examining the ferments and determining their absorption spectra, their "color," within the structure of the living cell, as it were, without separating them chemically from the other cellular substances. After the absorption spectrum of the ferment-CO combination had once been found, it was possible to compare it with the absorption spectra of substances which are accessible and can be analyzed by the ordinary methods of chemistry. In this way WARBURG (**12**) discovered that the constitution of the oxygen-transferring ferment of respiration in animal body cells and aerobic bacteria was phaeohaemin.

Neither alcohol fermentation nor lactic acid fermentation can be affected by carbon monoxide (**11**), and according to WARBURG and CHRISTIAN (**13**) "there are also oxidation processes which are insensitive to carbon monoxide as in *Chlorella*, yeast press juice and anaerobic bacteria." From these anaerobic bacteria it was possible to separate a catalytically active substance,

an iron-free oxidation ferment, the so-called yellow ferment of WARBURG (**13, 14**). So, at present we know the chemical constitution of two respiration ferments: the oxygen-transferring ferment, phaeohaemin, in animal cells and aerobic bacteria; and an oxygen-transferring ferment, the yellow ferment, in anaerobic bacteria.

PREVIOUS DATA ON EFFECT OF CARBON MONOXIDE UPON RESPIRATION OF PLANT CELLS

The influence of carbon monoxide upon the respiration of plant cells was studied by EMERSON (**3**), and PADOA and VITA (**8**). EMERSON'S experimental material was the alga *Chlorella*. As the result of his investigations EMERSON states that carbon monoxide has no effect upon the respiration of plants, which are autotrophic (*i.e.*, which obtain their energy by the "self-nourishment" of photosynthesis). Only after he succeeded in changing this original metabolism of plants into a heterotrophic metabolism, that is to say into a "parasitic" metabolism, by suspending the cells in a special sugar-containing nutrient medium, did the alga become sensitive to carbon monoxide. Under these conditions EMERSON found, in spite of a rather low partial pressure of oxygen and a high partial pressure of carbon monoxide (2.5 per cent. O_2, 97.5 per cent. CO; ratio $CO/O_2 = 39$), only a small inhibition of respiration (15 to 25 per cent.). The experimental material of PADOA and VITA (**8**) was *Plantago major, Lemna minor,* and *Elodea canadensis.* These authors report that the respiration of plants is not inhibited by CO, but that, on the contrary, CO increases their respiration.

Experimentation

The experiments discussed in this paper endeavor to answer the following questions: What is the chemical nature of the oxygen-transferring ferment of respiration in plants? Can this ferment be identified with one of the two oxidation ferments already known: the iron-free yellow ferment which is insensitive to carbon monoxide, or phaeohaemin which is sensitive to carbon monoxide? The writer has determined the effect of carbon monoxide on the respiration of the stamens of plums, stamens and pistils of daffodils, Spanish moss, green pine needles, and green leaves of tobacco, plum, and oleander plants. In all these plant cells the writer found that CO produces a strong reversible inhibition of respiration. For instance, in an atmosphere of 92 per cent. CO and 8 per cent. O_2 ($pCO/pO_2 = 11.5$) compared with an atmosphere of 92 per cent. N_2 and 8 per cent. O_2, the respiration of pine needles at a temperature of 20° C. is inhibited 70 per cent. This shows that the oxygen-transferring ferment of respiration in plants reacts with CO and must therefore be a compound of a heavy metal.

The inhibition of respiration by CO is reversible in visible light. By alternately illuminating plant cells and placing them in the dark in an atmosphere of O_2 and CO, respiration can be started or stopped. The ferment-CO compound dissociates in the light. This indicates that the oxygen-transferring ferment in plants is a colored substance and that it is a compound of iron.

The absorption of light by the respiration ferment-CO compound is of the same magnitude in plant cells as in liver cells and yeast. The rate of respiration of plum leaves, for example, in an atmosphere of $CO/O_2 = 12$, in the dark amounts to only 30 per cent. of the respiration in the CO/O_2 atmosphere when the leaves are illuminated by an ordinary 300-watt electric bulb placed 5 cm. below the bottom of the test vessel.

These findings indicate that the oxygen-transferring ferment of respiration in plant cells is identical with the oxygen-transferring phaeohaemin ferment of respiration in animal cells and aerobic bacteria. In white leaves (white ivy, *Zebrina pendula,* and sections of *Coleus*), petals, and roots, carbon monoxide did not have an effect of the same magnitude upon respiration.

Effect of carbon monoxide on respiration in the dark

Two to 10 young green tobacco leaves (dry weight 24 to 120 mg.), or 10 to 50 young green pine needles (dry weight 40 to 200 mg.) and similar amounts of plum leaves, Spanish moss, and oleander leaves were put into a conical manometer vessel. Into the insert well of the manometer was poured 0.2 cc. H_2O to supply the requisite humidity, and into the side arm 0.2 cc. of 8 per cent. KOH to absorb the carbon dioxide formed in respiration. The vessels were wrapped in tin foil to exclude light. Gas mixtures of oxygen and carbon monoxide, oxygen and argon, or oxygen and nitrogen were prepared over mercury in a 2-liter gasometer, and the manometer vessels were saturated with these gases. The oxygen consumption was measured at temperatures between 0° and 32° C. in the Warburg apparatus.

Figure 1 shows the respiration of tobacco leaves in the O_2 and N_2 gas mixtures and at various O_2 and CO concentrations; and figure 2 shows the rate of inhibition of respiration in pine needles at various CO/O_2 ratios. Even at a ratio $pCO/pO_2 = 4$ the respiration is inhibited by 38 per cent. At a ratio of $pCO/pO_2 = 20$ the inhibition amounts to 77 per cent. If oxygen-argon or oxygen-nitrogen mixtures are substituted for oxygen-carbon monoxide, the respiration rate again becomes the same as that of the control. The inhibition of respiration by CO is therefore totally reversible.

The effect of CO on respiration proved to be independent of temperature in tobacco leaves, pine needles, Spanish moss, and stamens of plums and daffodils. In pistils of daffodils (at $pCO/pO_2 = 20$) the rate of inhibition increased with increasing temperature (at 14° C. 16 per cent.; at 20° C., 38

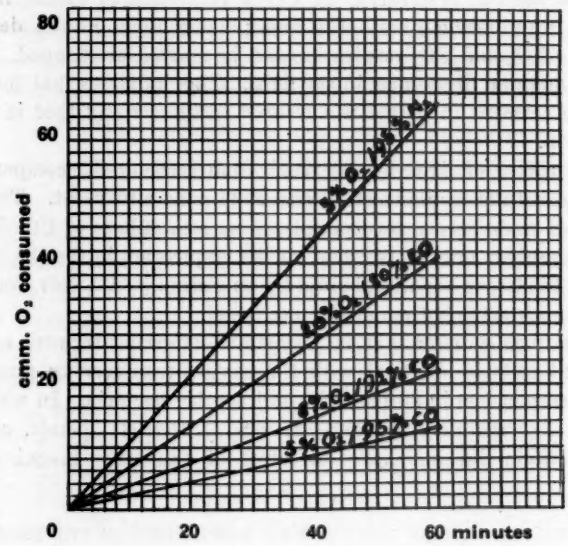

FIG. 1. Respiration of tobacco leaves (16 mg.) at various partial pressures of oxygen and carbon monoxide at 32° C.

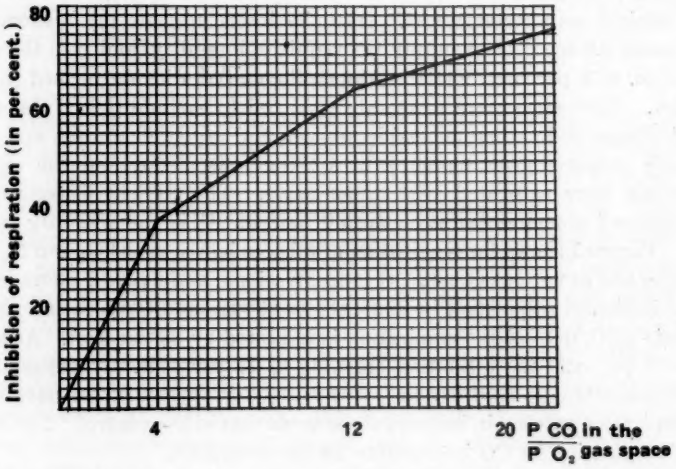

FIG. 2. Inhibitory effect of CO on respiration of pine needles at different ratios of CO/O_2 at 20° C.

per cent.; at 26° C., 53 per cent.) This dependence of CO inhibition on temperature is probably not due to a greater reactivity between the respiration ferment and CO, but rather to a different degree of saturation of the ferment at different temperatures under the experimental conditions.

Effect of carbon monoxide on respiration in light

The difficulty in measuring the effect of light on the CO inhibition of respiration in plants is obvious, because as soon as the green plant cells are irradiated by visible light photosynthesis begins. Even if no free carbon dioxide is available, as in the presence of KOH, the green cells form oxygen in the presence of light. This difficulty can be avoided by slightly narcotizing the green cells. As Bernard (**1**), and Bonnier and Mangin (**2**) found, small concentrations of chloroform stop the assimilation without decreasing the rate of respiration. Accordingly, 0.1–0.2 cc. of a 5 per cent. chloroform solution in alcohol was added to the test vessels containing green leaves of the higher plants or pine needles. The time required for complete narcosis of the assimilation depends upon temperature and the variety of plant cells. For instance, 4 to 6 hours, at temperatures of from 10° to 15° C., are necessary to obtain constant gas pressures with pine needles. After this

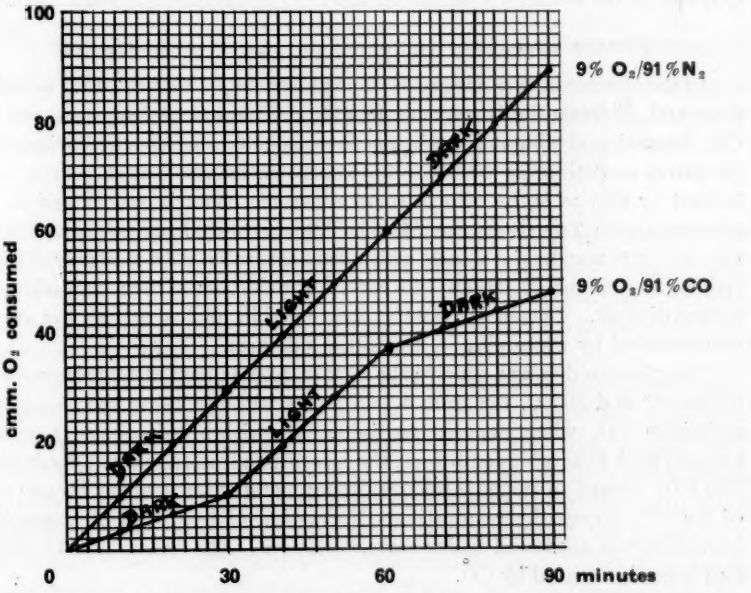

Fig. 3. Reversibility of carbon monoxide inhibition of respiraton of 120 mg. of pine needles in light at 10° C.

period, photosynthesis has ceased and the effect of light upon the CO inhibition of respiration can easily be measured. As source of light, a 300-watt electric bulb was placed 5 cm. below the bottom of the manometer vessel. The vessel was shaken in the thermostat at a temperature of 10° C. Figure 3 gives the result of an experiment which shows the effect of light upon the CO inhibition of the respiration of 120 mg. of pine needles in which assimilation was eliminated by chloroform. While the inhibition of respiration in the dark at a ratio of $pCO/pO_2 = 10$ was 67 per cent., the inhibition in the light was only 8.3 per cent. The combination of the respiration ferment with carbon monoxide can be entirely dissociated by visible light.

Effect of carbon monoxide upon respiration of tobacco leaves with mosaic disease

The effect of carbon monoxide on tobacco plants infected with mosaic disease has been studied in order to determine whether this virus disease changes the respiratory mechanism of the cells. Small young leaves and sections of leaves showing the spots of the mosaic disease were examined at various CO/O_2 ratios. Unless the cells had reached the stage of necrosis, no change was found in the rate of respiration, in the sensitivity of the respiration to CO, and in the sensitivity of the ferment-CO combination toward light.

Effect of carbon monoxide on plant fermentation

In the absence of free oxygen various plants can be kept alive for a limited time and photosynthesis may occur (**15**). The writer has measured the CO_2 formation of pine needles in the absence of free oxygen at various temperatures to determine whether this anaerobic metabolism in plants is inhibited by CO, as is the case in some anaerobic bacteria (**6, 9**) and in the photosynthetic Thiorhodaceae (**4, 5**). Thirty to 100 mg. of pine needles were put into manometer vessels which were saturated with mixtures of argon and carbon monoxide, argon and carbon dioxide, or carbon monoxide and carbon dioxide. To measure the CO_2 consumed in photosynthesis the oxygen was absorbed by white phosphorus in the side arm.

The pine needles can survive the lack of free oxygen at temperatures between 10° and 20° C. for from 4 to 6 hours without losing the capacity to assimilate CO_2 when exposed to light. If, within this time, they were brought back to aerobic conditions, the rate of respiration was not diminished. The CO_2 formed in the anaerobic-splitting metabolism is about 80 per cent. of the CO_2 formed by respiration. Formation of hydrogen in comparable quantities was not found under these conditions. The anaerobic CO_2 formation is not influenced by CO.

Since CO inhibits the respiration but not the anaerobic fermentation in plant cells, it is possible even under aerobic conditions to produce or inhibit

fermentation by adding or removing CO. Table I shows the effect of CO on the oxidative and on the splitting metabolism of 85 mg. of pine needles at 20° C. The O_2 absorbed and CO_2 released is measured in the presence and the absence of KOH in O_2 and N_2, O_2 and CO, N_2 and CO. The retention of CO_2 by the plant cells is measured by placing them in the insert well of the manometer vessel and determining the CO_2 which is liberated from a definite amount of bicarbonate contained in the bottom of the main vessel after adding citric acid from the side arm.

TABLE I

EFFECT OF CO ON THE OXIDATIVE AND THE SPLITTING METABOLISM OF 85 MG. OF PINE NEEDLES AT 20° C.

GASES	O_2 CONSUMED IN RESPIRATION	CO_2 FORMED IN RESPIRATION	CO_2 FORMED IN SPLITTING
	cu. mm.	*cu. mm.*	*cu. mm.*
8% O_2 – 92% N_2	100	100	None
8% O_2 – 92% CO	32	32	61
100% N_2	None	None	77
100% CO	None	None	77

The quotient, $\frac{CO_2 \text{ formed in respiration}}{O_2 \text{ consumed in respiration}}$, determined by this method is 1. While in an O_2 and N_2 atmosphere a non-oxidative metabolic process does not occur, in an atmosphere of O_2 and CO the CO_2 formed by splitting amounts to 80 per cent. of the CO_2 formed under anaerobic conditions.

Summary

1. The purpose of the work reported in this paper was to determine the chemical nature of the oxygen-transferring ferment in plants, and its possible identity with one of the two oxidation ferments already known, that is, with the iron-free yellow ferment which is insensitive to carbon monoxide, or phaeohaemin which is sensitive to carbon monoxide.

2. The effect of carbon monoxide on the respiration of stamens of plums, stamens and pistils of daffodils, Spanish moss, green pine needles, and green leaves of tobacco, plum, and oleander plants has been examined. It was found that the oxygen-transferring ferment of respiration in plants is a compound of a heavy metal, for in all these plant cells carbon monoxide produces a strong reversible inhibition of respiration, and instead of the respiratory metabolism a non-oxidative splitting metabolism appears. The oxygen-transferring ferment in plants is a compound of iron: for, the ferment-CO compound dissociates in light; the carbon monoxide inhibition of respiration

ceases if the cells are illuminated, and respiration again takes the place of the non-oxidative splitting metabolism.

3. The sensitivity of the respiration ferment toward carbon monoxide, and the absorption of light by the respiration ferment-CO compound are of the same magnitude in the cells of the higher plants as in animal cells and aerobic bacteria. All these findings indicate that the oxygen-transferring ferment of respiration in plant cells is identical with the oxygen-transferring ferment phaeohaemin.

The writer wishes to express cordial thanks to Dr. F. A. WOLF of the Department of Biology at Duke University for providing large quantities of the infected tobacco leaves for one of his experiments.

DUKE UNIVERSITY
DURHAM, NORTH CAROLINA

LITERATURE CITED

1. BERNARD, CL. Leçons sur les phénomènes de la vie. p. 278. Paris, 1878.
2. BONNIER, G., and MANGIN, L. Recherches sur l'action chlorophyllienne séparée de la respiration. Ann. Sci. Nat. (Botan.) 7th ser. **3**: 1–44. 1886.
3. EMERSON, R. The effect of certain respiratory inhibitors on the respiration of *Chlorella*. Jour. Gen. Physoil. **10**: 469–477. 1927.
4. GAFFRON, H. Über die Kohlensäureassimilation der roten Schwefelbakterien II. Biochem. Zeitschr. **279**: 1–33. 1935.
5. ————. Über die Unabhängigkeit der Kohlensäureassimilation der grünen Pflanzen von der Anwesenheit kleiner Sauerstoffmengen und über eine reversible Hemmung der Assimilation durch Kohlenoxyd. Biochem. Zeitschr. **280**: 337–359. 1935.
6. KEMPNER, W. Wirkung von Blausäure und Kohlenoxyd auf die Buttersäuregärung. Biochem. Zeitschr. **257**: 41–56. 1933.
7. ————, and KUBOWITZ, F. Wirkung des Lichtes auf die Kohlenoxydhemmung der Buttersäuregärung. Biochem. Zeitschr. **265**: 245–252. 1933.
8. PADOA, M., and VITA, N. Über die Wirkung von Kohlenoxyd auf frische Pflanzen. Biochem. Zeitschr. **244**: 296–302. 1932.
9. SCHLAYER, C. Wirkung des Kohlenoxyds auf die Gärung von Tetanus- und Gasbrandbazillen. Biochem. Zeitschr. **276**: 460–463. 1935.
10. WARBURG, O. Über die Wirkung des Kohlenoxyds auf den Stoffwechsel der Hefe. Biochem. Zeitschr. **177**: 471–486. 1926.
11. ————. Über die Wirkung von Kohlenoxyd und Stickoxyd auf Atmung und Gärung. Biochem. Zeitschr. **189**: 354–380. 1927.

12. ————————. Das sauerstoffübertragende Ferment der Atmung. Nobelvortrag. 1931.
13. ————————, and CHRISTIAN, W. Über ein neues Oxydationsferment und sein Absorptionsspektrum. Biochem. Zeitschr. **254**: 438–458. 1932.
14. ————————. Über das gelbe Ferment und seine Wirkungen. Biochem. Zeitschr. **266**: 377–411. 1933.
15. WILLSTÄTTER, R., and STOLL, A. Untersuchungen über die Assimilation der Kohlensäure. p. 344. Julius Springer. Berlin, 1918.

STIMULATION OF GROWTH OF SOY BEAN SEEDS BY SOFT X-RAYS

THEO. P. LONG AND H. KERSTEN

(WITH FIVE FIGURES)

Introduction

In recent years the effect of x-rays on the growth of seeds has been the subject of considerable investigation. Most of the work has been done with the Coolidge tube which, at the voltages commonly employed, produces chiefly hard, or short wave length x-rays, with only a small percentage of the energy of the radiation in the soft, or long wave length region of the x-ray spectrum. The injurious effect of large doses of any x-ray wave length seems established without contradiction, but the effect of small doses seems doubtful, producing sometimes stimulation as found by DOROSHENKO (**4**), INGBER (**7**), JOHNSON (**8**), NIKITIN (**15**), SNYDER (**16**), SHULL and MITCHELL (**17**); and again injury and retardation as found by ANCEL (**1**), CATTELL (**2**), DELONE (**3**), FRANCIS (**5**), GOODSPEED (**6**), JOHNSON (**10**), LALLEMAND (**12**), MALHOTRA (**13**), MOORE and HASKINS (**14**).

Greater stimulation was indicated when a filtered x-ray beam was used than when the x-rays were unfiltered in the work of NIKITIN (**15**), and SHULL and MITCHELL (**17**). Filters, such as those used in the experiments, reduce the intensity of all wave lengths but diminish the soft part of the spectrum preferentially so that if stimulation is obtained it may be attributed to the short wave length part of the x-ray spectrum and injury to the long wave length part, or, stimulation to the low intensity and injury to the high intensity, regardless of the wave length (**17**).

Since no experiments have been reported in which only soft x-rays were used to irradiate large numbers of dry seeds, it was thought desirable to carry out an experiment in which dry seeds were irradiated with only the soft part of the x-ray spectrum, and in which the effect of abnormal conditions upon the results was minimized by growing a large number of plants from irradiated and control seeds in permuted rows in the open field under natural soil and weather conditions.

Methods

Soy bean seeds were irradiated with soft x-rays produced by a copper target gas x-ray tube (**11**) operating at several different voltages ranging from 20 peak kv. to 35 peak kv. and 10 m.a. with the seeds 3.3 cm. from the focal spot of the x-ray tube. The most intense part of the x-ray spectrum consisted of the K_α (1.54 Å) and K_β (1.38 Å) lines of copper. The shortest

wave length was not less than 0.6 Å when 20 peak kv. were used and 0.35 Å when the voltage was 35 peak kv. See fig. 4.

Since the soft x-rays are absorbed appreciably by a few centimeters of air it was necessary to place the seeds near the window of the x-ray tube if they were to receive the maximum dose of soft radiation. In tubes of the type used the window is made of thin aluminum and cellophane which, in this case, was not strong enough to span an opening larger than three quarters of an inch in diameter. It was therefore necessary to make a machine which would place about 6 beans, oriented at random, under the window for 5 seconds and then eject them into a hopper and repeat the process (fig. 1). An exposure of 5 seconds was determined by preliminary experimentation as one which produced a proper minimum dosage. Larger doses were obtained by increasing the time of irradiation in steps of 5 seconds up to a maximum of 25 seconds. The time of irradiation was made accurate by the use of a synchronous motor driving a spiral gear and cam to operate the mechanism.

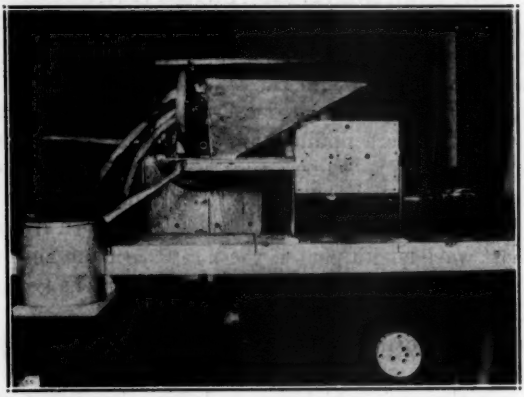

FIG. 1. Seed irradiating apparatus. White circle designates end of x-ray tube with window (not visible) on its lower face. X-ray beam is directed downward. Beneath the end of the x-ray tube is the machine which automatically removes seeds from the upper hopper, exposes them to x-rays for a predetermined time and then discharges them into the lower hopper.

The irradiated seeds were planted in trial plots. Each plot consisted of 36 rows, which were permuted in order that the variations in soil and moisture would tend to be averaged. Each row produced about 90 plants. The plants were harvested shortly after the first pods appeared (fig. 2), about 45 days after planting, by cutting them off at the surface of the ground. They were weighed immediately, to avoid loss of weight by evapor-

ation. The total weight of all plants having a certain time of irradiation, divided by the number of plants, was taken as the average wet weight.

Results

Five trials were made using dry Manchu soy beans irradiated at 20 peak kv. and 10 m.a. The total production was 12,751 plants. The results of each

FIG. 2. A typical plant at harvest time.

trial and the average of the five trials are shown in figure 3, in terms of percentage increase or decrease in wet weight of the irradiated beans compared with the wet weight of the control. The averages for the five trials were obtained by determining the arithmetical averages of all plants having the same period of irradiation.

Three additional trials were made using dry Wilson Black soy beans, following the same procedure as that used for the Manchu soy beans except that for one trial the seeds were irradiated at 25 peak kv., for another at 30 peak kv., and for a third at 35 peak kv.

When the voltage across an x-ray tube is increased the intensity of each wave length, and hence of the total beam, is increased approximately proportional to the square of the voltages applied; also, more of the harder radiation is included in the beam, as figure 4 illustrates. The shortest wave length emitted at the voltages used in irradiating the Wilson Black soy beans was approximately 0.50 Å, 0.40 Å, and 0.35 Å respectively. A total of 13,824 Wilson Black soy bean plants were produced in the three trials. The results are shown in figure 5.

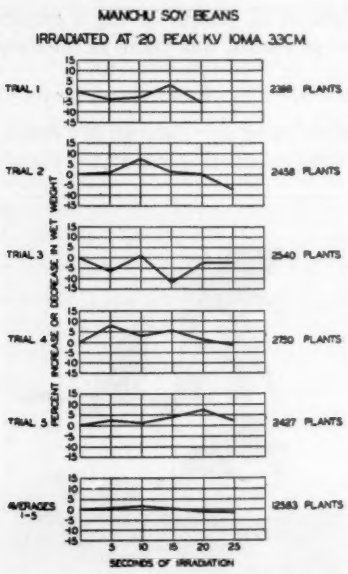

FIG. 3. Results of the first five trials.

Discussion

Results varied considerably for the same dosage in the different trials. After the results had been obtained it was supposed that this might have been due in part to the random orientations of the beans when they were being irradiated. Experiments were then conducted on a smaller scale. The beans were placed with their embryo-sides toward the source of x-rays in one set, and in another set with their embryo-sides away from the source of x-rays, and therefore shielded from the x-ray beam by their cotyledons. Each set was given the same dose of x-rays, which was sufficient to cause injury but it was observed that the plants grown from the set whose embryo-sides had been placed toward the source of x-rays were injured in a greater degree than plants grown from the other set. This difference is probably due to the fact that the embryo is more easily injured by the x-rays than the cotyledon, and because soft x-rays are absorbed so greatly by the substance of the bean seeds that an embryo shielded by the cotyledon would receive a very much smaller dose than another embryo exposed to the same dose but not so shielded. If all seeds were oriented in the same way they should each receive approximately the same effective dosage and a greater average variation from the control would be expected, either in the form of greater stimulation or greater retardation. This possibility is being further investigated by the writers.

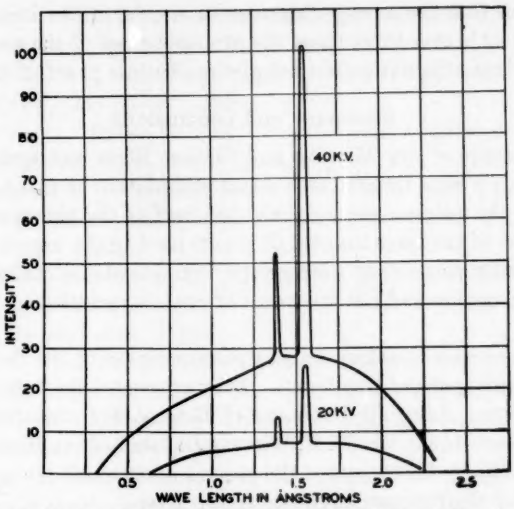

FIG. 4. Probable distribution of intensities of emitted radiation in the x-ray spectrum when the tube is operated at the indicated voltages. Illustrates nature of changes in composition of beam when voltage is changed.

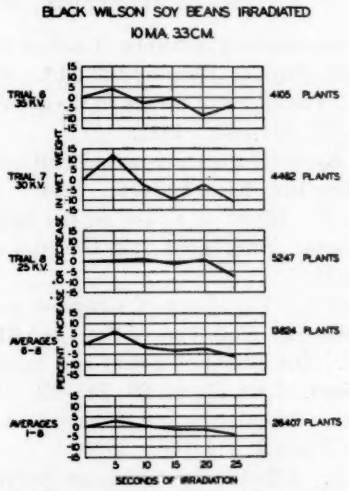

FIG. 5. Results of the last three trials and averages of all the trials.

Because of the large number of plants produced and the conditions under which they were grown the average results obtained seem to represent a true effect of the radiation and not chance variation. Statistical treatment of the

data indicates that the average increase in weight of the irradiated plants over the control is over three times the probable error of the average thereby indicating a true stimulation but not giving absolute proof of it.

Summary and conclusions

1. Irradiation of dry Manchu and Wilson Black soy beans with small dosages of soft x-rays resulted in a slight stimulation of plant growth when measured by the average wet weight of the part of the plants above ground.

2. Because of the large number of plants used in the experiment and the conditions under which they were grown, the indicated stimulation seems to be a true stimulation and not the result of chance variations.

The authors wish to acknowledge the cooperation of the Botany Department of the University of Cincinnati. They are especially indebted to Dr. J. HOBART HOSKINS, Associate Professor of Botany, for consultation and advice. Thanks are due to Mr. W. E. WEAVER and staff of the Hamilton County Experimental Farm for the use of the ground upon which the soy beans were grown, and for their expert advice so freely given.

UNIVERSITY OF CINCINNATI
CINCINNATI, OHIO

LITERATURE CITED

1. ANCEL, S. The accelerating influence of x-rays on the development of plants. Arch. Physiol. Biol. **5**: 106–118. 1929.
2. CATTELL, WARE. The effects of x-rays on the growth of wheat seedlings. Science, n.s. **73**: 531–533. 1931.
3. DELONE, L. N. Experiments on x-raying cultivated plants. Contrib. Plant Breeding Inst. Kiev. **4**: 3–16. 1928.
4. DOROSHENKO, A. V. Effect of x-rays on the length of the vegetation period in plants. Bull. Appl. Bot. Genetics and Plant Breed. **23**: 511–535. 1930.
5. FRANCIS, DOROTHY S. The effects of x-rays on growth and respiration of wheat seedlings. Bull. Torrey Bot. Club **61**: 119–153. 1934.
6. GOODSPEED, T. H. The effects of x-rays and radium on species of the genus *Nicotiana*. Jour. Hered. **20**: 243–259. 1929.
7. INGBER, EDMONDO. Stimulation and damage by x-rays. Alli. Ist. Bot. Ital. R. Univ. Pavia **2**: 111–115. 1930.
8. JOHNSON, EDNA L. Effects of x-rays upon growth, development, and oxidizing enzymes of *Helianthus annuus*. Bot. Gaz. **82**: 373–402. 1926.
9. ————————. Growth and germination of sunflowers as influenced by x-rays. Amer. Jour. Bot. **15**: 65–77. 1928.

10. ————————. On the alleged stimulating action of x-rays upon plants. Amer. Jour. Bot. **18**: 603–614. 1931.
11. KERSTEN, H. A. Gas x-ray tube for irradiation with soft x-rays. Radiology **23**: 60–63. 1934.
12. LALLEMAND, S. Étude de l'action des rayons x sur le développement des plantes. Arch. Anat. Hist. Embryol. **10**: 1–233. 1929.
13. MALHOTRA, R. C. The influence of ultra-violet rays, x-rays, and temperature on the germination of *Zea mays*. Jour. Indian Bot. Soc. **11**: 1–27. 1932.
14. MOORE, C. N., and HASKINS, C. P. Physiological variations in certain crop plants following seed exposure to high-voltage x-rays. Bot. Gaz. **94**: 801–807. 1933.
15. NIKITIN, I. Breeding and introduction of rapidly growing tree plants. The U.S.S.R. Central Forestry Res. Inst., Bull. no. 1. 1934.
16. SNYDER, G. E. Effect of x-rays on developing corn. Proc. Pennsylvania Acad. Sci. **4**: 32–34. 1930.
17. SHULL, C. A., and MITCHELL, J. W. Stimulative effects of x-rays on plant growth. Plant Physiol. **8**: 287–296. 1933.

622

THE STATE OF WATER IN DUCTS AND TRACHEIDS

GEORGE J. PEIRCE

In preceding papers (**3, 4, 5**) I have discussed various aspects of the problem of the ascent of sap, of how water goes up a tree, and I wish in this paper to carry the discussion forward another step. First, however, I wish to express certain convictions, the fruits of observation, experiment, and reflection, under climatic conditions of considerable variety.

I. If one conceive the living body to be a continuous mass of water, then one will readily admit the undesirability of interrupting the mass by wound or amputation in any study of water movement, and the possibility that the evidence produced after or in connection with the injury may be misleading. Therefore in recent experiments I have avoided all mechanical injury, even to the extent of using undisturbed trees, etc., out of doors. Shocks from uprooting, transplanting; diminished absorption through injured roots; cells in phloem and xylem stimulated to unusual if not abnormal activity by cutting or pressure; change in exposure to light, air, etc., following transplanting, or the transfer of potted plants to other positions; all of these have been avoided, as previously described, by using trees *in situ* growing out of doors. STRASBURGER (**7**) in his famous experiments on trees in the Botanical Garden in Bonn used living trees, but he amputated them at the butt. I have in nowise disturbed my trees except by applications to limited and intact parts, and by the results of these applications in the parts beyond.

II. It is absurd that it should be necessary to point out that glass and cellulose, and the derivatives of cellulose found in the bodies of plants, bear very different relations to water; and to warn the student that the results of experiment with glass may be quite misleading as to what actually occurs in cellulose and cellulose derivatives. It is true that the adhesion between water and *clean* glass is considerable; but anyone knows that to dry glass in any form to a constant weight is a task easier and quicker than to dry any mass of cellulose, whether lumber or fabric. The relations of water and cellulose, furthermore, are not merely mass relations, as for the most part at least between water and glass; but they are molecular relations, and there is in every cellulose mass a certain amount of *bound* water. It was with realization of this that WOODHOUSE (**8**) tried to qualify and correct his experiments in the glass tubing which he used. But in my experiments I have avoided the use of glass and of all supposed imitations of plant structures.

III. I have lived for many years in a semi-arid climate, in which the temperatures at all seasons are moderate, in which there are regularly

alternating rainy and dry seasons during which atmospheric humidities are respectively high and low; and illumination is more intense and richer than in corresponding latitudes and altitudes with which I am acquainted elsewhere. Just as the sugar maple has a season in which it can be bled of its sweet sap, and another much longer season in which no sap will flow from it, so we have plants which exhibit similar phenomena, with little or no difference in the water maxima, but with water deficits more pronounced and even revealing themselves by wilting, by rainy season and dry season foliage, by leaf fall, and by complete disappearance from the landscape. I have seen *Pinus radiata* Don. wilt in sandy soil near the coast under high temperatures on dry sunny days. The tarweeds (*Hemizonia*, etc.) have dry season foliage made up of relatively few long, narrow, and glandular-hairy leaves borne on the slender woody flowering stem, which rises in late spring from a rosette of broad, rather fleshy and smooth leaves, which has formed on the surface of the damp soil during the rainy season. *Aesculus californica* Nutt., the California buckeye, is our first tree to put out leaves in the early spring (February or March or April) and the first to lose them in early summer (in June or July or August), according to the dryness of the soil in which it grows; and the Miners' Lettuce, of the days of the "Gold Rush," *Montia perfoliata* Howell, thriving in shade, commonly under oaks, and in orchards, during the rainy season, dries and dies at the beginning of the dry season. This last is as characteristically a rainy season plant as the hayfield tarweeds in bloom are characteristic of the dry season. Their vascular tissues correspond to their respective seasons of growth, their heights and their foliage (3).

Under these conditions the maintenance of solid columns or cylinders of water in ducts and tracheids would seem to be difficult and doubtful. This is recognized by MacDougal, Overton, and Smith (1) who give the name of the pneumatic system to those ducts and tracheids in the plants which they studied which do not contain liquid. That there are such elements in the vascular bundles cannot be questioned; but the relative numbers of hydrostatic and of pneumatic elements at one time is not necessarily the same at all times, as may be demonstrated by testing with starch suspension (6). The elements filled with water are obviously most numerous at times of water surplus, fewest at times of water deficit. The same set of ducts or tracheids will, therefore, be a part of the hydrostatic system at one time and of the pneumatic system at another. When they are full of water they may be made to bleed; when they cannot bleed, what do they contain?

This question is only partly answered in MacDougal's later paper (2), for while the proportions of carbon dioxide, oxygen, and nitrogen are reported, there is no indication of the proportion of water vapor. Since it

is inconceivable that the contents of the pneumatic system form a mixture of *dry* gases, water vapor must be present, varying in proportion from time to time, as do the proportions of the pneumatic system itself.

From the foregoing it is clear that in the mass of wet wood which constitutes a part of the vascular system of a living land plant, water may exist exclusively in liquid form, or part of it in liquid form, part of it as vapor. The liquid water may *fill* a duct or a tracheid, it may be "bound" in the walls, it may be more or less free on the surface of the walls as a thicker or thinner film, and it may fill as vapor the remainder of the space surrounded by the wet wall. If this reasoning and these inferences are correct, it is possible to prove them by proper experiment.

If one apply sufficient cold to the outside of an intact branch of a living plant, whatever water is in the part chilled will condense to liquid and will freeze. If the quantity of water be sufficient to form an ice plug in the vessel, movement of any sort through that part of the vessel will be prevented by the plug. If, on the other hand, the quantity of water be insufficient to close the vessel, motion through it will not be prevented. It is possible to blow through certain kinds of wood when they are dry, notably oak. Of course it is not possible to blow through the tracheids of the wood of the Coniferæ. Branches of California buckeye can be blown through as readily as oak branches or pieces of oak wood because of the large ducts in the xylem. One may blow through dry pieces of buckeye wood, demonstrating the results by bubbling through water or by moistening and soaping the end through which the gas exudes. If one use breath, bicycle tire-pump, or compressed air, the result is the same in kind—sooner or later bubbles come through. They come through immediately in the dry season, less and less promptly the earlier in the season the experiment is made; for the more water there is in the wood, the more must be blown out before bubbling can occur. But if the wood should be full of water and frozen by liquid air, as described in a previous paper (5), air could not be blown through as long as the water remained frozen. On the other hand, if the wood should contain little more liquid water than that bound in the walls of ducts and tracheids, freezing would not cause water plugs to form in the ducts, and air could be blown through.

The experimental record is as follows:

April 11, 1935, 3 P.M. Into a paper cup made as described previously (5) pour liquid air to the depth of 1 cm. (approximately) around a leafy branch of buckeye, near the laboratory.

3:15 P.M. After thorough freezing cut off the branch below the frozen zone, rush into the laboratory, carrying branch with butt in Dewar flask containing liquid air in the bottom; cut fresh surface, con-

nect with compressed air cock, open the cock and attempt to blow through, with the end under water: *no bubbles.*

But yesterday a similar branch, cut off but not previously frozen, *frothed* when tested with compressed air. This showed the presence of continuous open ducts for lengths of 15 cm. or more.

At these dates there had been a total rainfall of 16″ for the season (since the preceding July 1), soil and vegetation were full of water, and there should at this time, if ever, be continuous columns of water in some or all ducts. Hence I expected the vascular system at this time to be full of water and therefore plugged with ice if I applied sufficient cold. This was the case as the observation showed.

April 12, 2 P.M. Put two paper cups on two erect foliage branches of buckeye, tying tight with adhesive tape at the bottom of the cups, and pour liquid air into the cups to freeze, not chill, a zone of 1 cm. on each branch. Air temperature 20° C., sunshine and clouds.

Within 5 minutes, the foliage above the cups wilting.

" 10–15 minutes leaflets and tender shoots hanging limp.

April 17, 11 A.M. Air temperature 16.5° C. No recovery of foliage of frozen branches, leaves dead, dry, though much cloudy weather, 0.7 in. rain, high humidity and little full sunshine since preceding entry, and the rest of the foliage of the tree in fine condition.

May 2, 10:35 A.M. Liquid air treatment, amputation, and compressed air as above: *no bubbling.*

Air temp. 17.5° C., humidity high, soil moist from recent rain. Longitudinal halving of the branch showed that the water continued to move from above the frozen (plugged) part toward the foliage, and that the part of the vascular system just above the frozen zone, under these conditions, was dried by the withdrawal of its water by the parts above.

May 20, 3:40 P.M. Air temp. 32.4° C., top leaves of buckeyes half wilted, but no pronounced water deficit further down as shown by freezing and using compressed air on the green current year's branch, through which slight bubbling was noted. Same treatment of a last year's branch, bearing new growth and new foliage beyond, showed *no bubbling.* My work was interrupted and delayed soon after; but later in the season, and before the leaves had fallen, when, therefore, there should be the maximum draft upon the conducting tissues by reason of the low humidities, at least by day, the warmth and motion of the air, the drying of the soil because of the absence of rain from the onset of the dry season, freezing and compressed air showed the passage of air, as indicated by bubbles, through the branches tested. These internodes were 15–20 cm. long. By using internodes, anastomoses at the joints and at leaf bases were avoided.

It may be said that this compressed air treatment after freezing shows only that there are unfilled (unplugged) vessels in the vascular system of buckeye, that it does not show that there are no vessels filled with solid columns of water at normal temperatures. This is true; but it is also true that, with the change from a water surplus to a water deficit, the freezing-compressed air treatment shows a corresponding change from no passage of air, to the passage of small amounts of air, and of considerable air, through the vascular system. The proportions of hydrostatic to pneumatic tissues in the vascular system are not fixed, but vary from time to time according to the internal and external circumstances of a tree (3), there being very little if any pneumatic tissue at the time when bleeding is a natural or induced phenomenon, there being very little if any hydrostatic tissue containing water other than as vapor, film, and bound water at times of deficit, when bleeding is not possible.

This is confirmed by repeated observation that, in buckeye, castor bean, and other plants, the position of starch suspension (6) used to show the course of water movement varies according to the water relations of the plant upon which one experiments. At times of water abundance some, but not necessessarily all, vessels may be *filled* with starch suspension. At times of less and less water, the starch suspension is confined more and more to the walls of the vessels, *except* in the region immediately affected by the injection following amputation of the branch under the surface of the starch suspension.

It was the repeated observation of this distribution of starch in the vascular system of the plants experimented upon by using the starch suspension method that led me to consider how to determine, without amputation, the condition and the position of the water in the vessels at different times, and to conclude that, instead of the water being at all times in a larger or smaller number of unbroken columns of liquid, it may be in unbroken liquid columns or in hollow liquid columns (films on the walls) filled with water vapor, and water bound in the cellulose and cellulose derivatives of the walls. As indicated above, the application of freezing cold to short zones of water-conducting intact branches confirms the conclusions suggested by the condition in amputated branches as shown by the starch suspension method.

I feel, therefore, that the idea as to the condition and movement of water, as set forth in my first paper (3) is correct as regards woody plants in arid and semi-arid areas, and in all vascular plants at times of water deficit in the vascular tissues, whatever may be the condition at times of water surplus in humid climates whether tropical or temperate. That there may be great force (suction force) exerted by the foliar parts above upon the conducting vascular tissues below is obvious: but that this force

is dependent upon micro-menisci in the walls of mesophyll cells does not seem necessary or indicated by the evidence. Evaporation, transpiration from the surfaces, wherever they may be, of the continuous mass of water of which each living organism consists, will set up in the mass forces, tensions, which will conform in place and power to the structure and composition of the system. The bound water of the walls of the vascular elements forms a water-seal which obviates and excludes drawing in air from the sides. Hence the traction is upward, not lateral; it is a lifting force pulling up the water as vapor or as liquid, according to position and condition in the vascular system. That this force is adequate has been shown repeatedly by others.

The maintenance of water as vapor, liquid film, and liquid mass in the vascular elements and their walls, in the hydrostatic-pneumatic system of woody plants, is possible only when living cells are duly associated with the vascular tissues. Death of these cells by poisons and by heat, immediately interfering with the moving mass of water only to a partial extent, disturbs the movement of sap far less than cold, which completely stops the movement of the water as well as the life of the cells in the zone of freezing. The living cells are indispensable, not for moving, but for conditioning the movement of the water.

LITERATURE CITED

1. MacDougal, D. T., Overton, J. B., and Smith, G. M. The hydrostatic-pneumatic system of certain trees: movements of liquids and gases. Carnegie Inst. of Washington, Publ. no. 397. 1929.
2. ————, and Working, E. B. The pneumatic system of plants, especially trees. Carnegie Inst. of Washington, Publ. no. 441. 1933.
3. Peirce, G. J. Observations on sap hydraulics. Amer. Jour. Bot. **21**: 211–227. 1934.
4. ————. The ascent of sap. Madroño **3**: 1–3. 1935.
5. ————. Are living cells involved in the ascent of sap? Amer. Jour. Bot. **23**: 159–162. 1936.
6. ————. Experimental plant physiology. pp. 61–62. New York. 1931.
7. Strasburger, E. Ueber das Saftsteigen. Jena. 1893.
8. Woodhouse, E. D. Sap hydraulics. Plant Physiol. **8**: 177–202. 1933.

APPROXIMATE GERMINATION TEST FOR NON-AFTER-RIPENED PEACH SEED[1]

H. B. Tukey and M. S. Barrett

(WITH ONE FIGURE)

Introduction

Following the severe winter of 1933 and 1934, with its attendant losses of orchard trees from winter cold, the demand increased sharply for nursery trees for replanting. A shortage of peach trees resulted. The situation was made acute, however, by the poor germination of peach seed planted by nurserymen, for propagation purposes, to meet the demand for trees.

Nurserymen commonly plant peach seed in the field in the fall of the year and bud the resulting peach seedlings to the desired variety the following summer. In one instance, fairly typical of a large percentage of the nursery industry, sufficient acreage was planted to peach pits in the fall of 1933 to produce 200,000 seedlings for propagation purposes, but only 16,000 developed. Some rapid test of peach seed germination prior to fall planting would, therefore, be of immediate practical aid to the nursery industry.

Unfortunately, peach seed, in common with seed of many rosaceous plants, requires a period of after-ripening before it will germinate. For peach seed the after-ripening period is 12 weeks (3) at temperatures slightly above freezing (5° C.). Such a test is impractical for the current season's seed supply for fall planting.

From favorable responses in artificial culture of excised embryos of deciduous fruits (4) the suggestion was secured that an approximate test of viability and germinability might be obtained by this method. Crocker and Barton (1) reported more than 50 per cent. germination of peach seed when the peach embryos are entirely removed from the coats and placed on moist filter paper at room temperature. Flemion (2) secured 80 to 90 per cent. germination of naked, non-after-ripened peach embryos after 5 to 7 days in moist peat moss at 25° C.

Materials and methods

Peach pits[2] were obtained from North Carolina, Virginia, Kentucky, Tennessee, California, New York, and Japan. In addition, samples were

[1] Approved by the Director of the New York State Agricultural Experiment Station as Journal Paper no. 135.

[2] Nurserymen commonly speak of peach pits incorrectly as peach "seeds," whereas the stony pericarp is part of the fruit and encloses the seed.

received from various commercial nurseries in Missouri, Michigan, Ohio, Iowa, and New York, giving a total of 73 different samples and several thousand seeds that were tested during two seasons.

Peach pits were cracked, the seeds soaked for several hours in water, and the seed coats removed together with any small amount of nucellar tissue and endosperm. Square ½-ounce glass bottles with wide mouth and metal screw caps were used as containers, and were filled with 10 cc. of agar media of various concentrations, and containing various nutrients. The bottles were autoclaved for 20 minutes at 15 lb. pressure and promptly cooled in order to prevent acid hydrolysis of the media.

The nutrients used were those contained in Knop's formula, Crone's nitrogen-free formula, and a formula developed in the laboratory of the New York State Agricultural Experiment Station for embryo culture work (4). To some of these were added ½, 1, and 2 per cent. glucose, and to others 2 per cent. sucrose. Agar concentrations were varied from ½ to ⅔, 1, and 2 per cent.

FIG. 1. Response of two samples of naked, non-after-ripened peach embryos on ⅔ per cent. agar in artificial culture. Above, cotyledons spread, root and shoot developing, and 100 per cent. germination; below, no response and no germination.

Naked embryos were sterilized for 30 minutes in calcium hypochlorite solution (5). They were introduced, by means of flamed wire loops, into the bottles from the chlorine solution without rinsing. The bottles were closed tightly and kept at temperatures of 15°, 20°, 22°, 25°, 30°, and 35° C.

The responses of the naked embryos to this culture method were recorded as "cracked" when the cotyledons separated a millimeter at the tips; "open" when the cotyledons were spread at an angle of 75 degrees or more to each other; and "growing" when the epicotyl developed into a shoot and the hypocotyl into a root.

For comparison, other lots of peach pits were cracked, and the seeds removed and placed intact in moist peat moss in a refrigerator at 5° C. Each week, for 15 weeks, examinations were made, and seeds which showed signs of germination were counted and removed. Most germination occurred from the tenth to the twelfth week under these conditions.

Field observations also were made, in which the germination figure, obtained by the culture method before planting, was compared with the actual results secured in the nursery from several hundred thousand seeds.

Results

The results from the culture method, with several typical lots of peach seed in comparison with the after-ripening germination test, are given in tables I and II. They agree essentially with the results from other samples.

TABLE I

COMPARISON OF CULTURE METHOD AND AFTER-RIPENING METHOD FOR TESTING PEACH SEED GERMINATION

SOURCE	NUMBER OF SEEDS	PERCENTAGE OPEN OR GROWING WHEN CULTURED ON AGAR 7 DAYS	PERCENTAGE GERMINATION WHEN AFTER-RIPENED 10 TO 15 WEEKS AT 5° C.
		%	%
Nursery A	254	67.8	64.6
" B	62	36.2	25.0
" C	60	10.0	00.0
" D	228	80.0	73.9
" E	545	00.0	00.0
Seedsman A	67	100.0	85.7
" B	94	10.0	16.6

It will be noted that in most cases the percentage for the culture method is slightly higher than the percentage for the after-ripening method. This is, in part, due to the more favorable conditions provided by the culture

TABLE II

COMPARISON OF TEST FOR PEACH SEED GERMINATION BY CULTURE METHOD AND COMMERCIAL FIELD GERMINATION

SOURCE	NUMBER OF SEEDS	PERCENTAGE OPEN OR GROWING WHEN CULTURED ON AGAR 7 DAYS	NUMBER	PERCENTAGE GERMINATION FROM FALL-PLANTED COMMERCIAL PLANTINGS
		%		%
Nursery A	32	16	Ca 25,000	Ca 17
" B	20	67	Ca 125,000	Ca 75
" C	22	0	Ca 25,000	0

methods. Many instances were recorded of slight viability as shown by separation of the cotyledons in culture but which failed to appear in germination records by the after-ripening test. In all probability some of the embryos noted as open were border-line cases and this increased the percentage by the culture method. Embryos should be counted as germinable in culture only when they show sufficient vigor to spread the cotyledons at an angle of 75 degrees and begin shoot and root development.

Discussion

While this method may be considered only approximate it gives figures which bear a direct relation to the after-ripening test, and it serves to meet the practical need for a rapid test of germinability. The method is suggested as having possibilities in meeting other problems of similar nature.

There seemed to be neither advantage nor disadvantage in the use of the materials comprising the various media, excepting that glucose and sucrose showed an inhibiting effect upon development of cultured embryos. Absence of nutrients in the media is an advantage because it reduces the possibility of the development of contaminating organisms. Of the agar concentration, $\frac{3}{4}$ per cent. seemed to give best results. This concentration is sufficient to provide support for the embryos and yet not so much as to reduce the available moisture.

Room temperatures of 18° to 22° C. were satisfactory. Temperatures of 30° to 35° C. hastened development, and temperatures of 15° C. delayed development.

The results of the field tests given in table II agree quite closely with the culture methods, and substantiate the laboratory comparisons given in table I. These figures were obtained from counts by commercial operators

involving approximately 150,000 seeds that were planted in the fall and handled by commercial methods.

Summary

1. To meet an emergency an approximate germination test is reported for peach seed obtainable in 7 days as compared with the normal after-ripening method of 10 to 12 weeks.

2. Naked embryos, sterilized in 2 per cent. chlorine solution for 30 minutes and placed in sterilized ½-ounce bottles on ⅔ per cent. agar medium showed varying degrees of response; namely, (A) no response, (B) cotyledons slightly spreading, (C) cotyledons spread at an angle of 75 degrees, and (D) shoot and root growth developed.

The degree of response in C and D taken together gives a figure which approximates closely the figure for germination secured by after-ripening similar samples in damp peat moss at 5° C. for 12 weeks.

3. Various nutrients and agar concentrations other than ⅔ per cent. either failed to improve the response or else proved to be inhibiting.

4. The percentage of seeds germinated from fall plantings under nursery conditions agrees closely with the figures secured by the culture method prior to planting.

NEW YORK AGRICULTURAL EXPERIMENT STATION
GENEVA, NEW YORK

LITERATURE CITED

1. CROCKER, WILLIAM, and BARTON, LELA V. After-ripening, germination, and storage of certain rosaceous seeds. Contrib. Boyce Thompson Inst. **3**: 385–404. 1931.
2. FLEMION, FLORENCE. Dwarf seedlings from non-after-ripened embryos of peach, apple, and hawthorn. Contrib. Boyce Thompson Inst. **6**: 205–209. 1934.
3. TUKEY, H. B. Studies of fruit seed storage and germination. New York Agr. Exp. Sta. Bull. 509. 1924.
4. ————. Artificial culture methods for isolated embryos of deciduous fruits. Proc. Amer. Soc. Hort. Sci. **32**: 313–322. 1935.
5. WILSON, J. K. Calcium hypochlorite as a seed sterilizer. Amer. Jour. Bot. **2**: 420–427. 1915.

STERILIZATION OF CORN GRAINS WITH SODIUM HYPOCHLORITE

RAYMOND E. GIRTON

(WITH FOUR FIGURES)

Introduction

Before carrying out a respiration study on detached roots, it was necessary to work out a method for obtaining root tissues free from microorganisms. This was essential because fungi give off considerable carbon dioxide in respiration; and the production of respiratory carbon dioxide by asterile roots may be entirely masked by the presence of these microorganisms.

Following the method outlined by WILSON (**4**), an aqueous extract was prepared from bleaching powder. Corn grains were treated with this solution, and allowed to germinate on sterile agar, as employed in the method of ROBBINS (**2**). Other grains were treated with a ¼ per cent. organic mercury preparation, and germinated on sterile agar. Neither of these methods proved to be satisfactory. Since roots which grow from seed sterilized with the organic mercury solution frequently become infected after being detached from the seed, it was assumed that some of the microorganisms were not killed, but were merely inhibited by the disinfectant. A volatile disinfectant, sodium hypochlorite, was therefore chosen because it would either kill the fungi and bacteria on the corn grains during the period of treatment, or allow the subsequent growth and detection of the microorganisms before the roots were detached. DUGGAR and DAVIS (**1**) included sodium hypochlorite in their list of substances tested as seed disinfectants. They used solutions made up from solid (?) sodium hypochlorite, and obtained favorable results which, however, were not altogether uniform.

Preliminary procedure

STANDARDIZATION OF DISINFECTING SOLUTION

A commercial preparation of sodium hypochlorite in solution was obtained. The effective chlorine strength of this solution was determined by a method outlined by TREADWELL and HALL (**3**). This method is based upon the assumption that the oxidation of combined iodine to free iodine is brought about by the chlorine in the disinfecting solution. The iodine thus released is titrated with sodium thiosulphate. Starch is used as the indicator.

The free chlorine of the commercial hypochlorite solution was estimated as 3 per cent. An appropriate dilution of this solution reduced the chlorine concentration to 1 per cent., which was an effective strength for the sterilization of corn grains.

Sterilization of grains

The sterilization procedure consisted in treating about 100 grains of corn in a stoppered flask with 50 cc. of the disinfecting solution. The length of treatment was from 0.5 to 5 hours. During this period, the flask was continuously shaken to promote thorough mixing of the solution and effective sterilization.

At the end of the treatment, the stopper was partially removed and the hypochlorite solution drained off. The grains were then transferred, by means of a long handled metal spoon, to Petri dishes which contained sterile agar. The spoon was frequently dipped into alcohol and then put into a flame in order to free it from microorganisms. As an added precaution against introducing contamination, these transfers were always made in an inoculation chamber.

Germination of grains

Following their transfer to agar plates, the corn grains were germinated in darkness at, or near, room temperature for a period of 5 to 7 days. Daily examinations were made, without removing the Petri-dish covers, and a record was taken of the germination and number of infected grains. The germination of corn grains, as the result of this treatment, is shown in figure 1. In this case, 8 of 10 grains had germinated. None gave evidence of being contaminated with microorganisms.

Fig. 1. Sterilized corn grains germinated under aseptic conditions.

Experimentation

Effect of varying time of treatment

Several experiments were carried out to determine the effect of different lengths of treatment. Two of these experiments will be described. In both cases the Krug variety of yellow dent corn was used. The corn was furnished by Mr. John Trost of the Purdue Agricultural Experiment Station. The disinfecting solutions contained 1 per cent. chlorine. This was checked by titration.

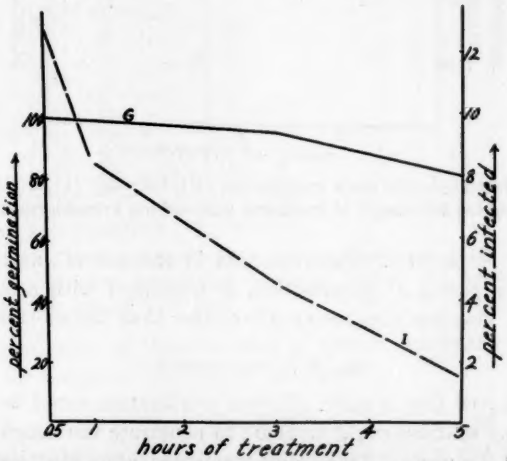

Fig. 2. Effect upon germination and infection of corn grains of varying the length of treatment with sodium hypochlorite solution.

In the first experiment, four lots of 70 grains each were sterilized for 0.5, 1, 3, and 5 hours, respectively. The results of this experiment are shown in figure 2. From this graph it can be seen that increased time of treatment somewhat depressed the germination, and markedly decreased the number of grains which developed infection during the 5-day germination period. The 100 per cent. germination achieved by the grains sterilized for but 0.5 hour fell to 80 per cent. when the sterilization period was lengthened to 5 hours. At the same time, however, an infection of 13 per cent. of the grains which developed with the shortest treatment was reduced to less than 2 per cent. for the longest period.

The second experiment (fig. 3), in general, confirmed the results of the first experiment. Five lots of 100 grains each were sterilized for periods of 1, 2, 3, 4, and 5 hours. Two curves of the graph show that greater duration of treatment over a 5-day period, serves to depress germination (G), and greatly to reduce the number of infected grains (I). A third curve of the graph shows the effect of the longer periods of treatment in the reduction

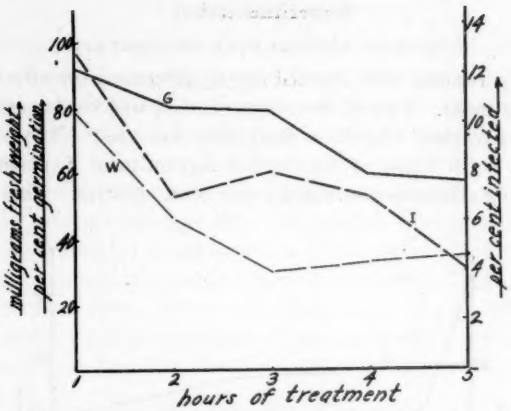

FIG. 3. Effect upon corn grain germination (*G*), infection (*I*), and fresh weight of roots (*W*), of varying the length of treatment with sodium hypochlorite solution.

in size of the roots (*W*). The reduction in the size of the root, attained within a 5-day period of germination, is associated with a slower rate of germination. This was also observed for other than the shortest treatments.

SUCTION TREATMENT

It was believed that a more effective sterilization could be obtained, if the disinfecting solution could be made to penetrate the rough loose tissues at the base of the grain. This belief was based upon the observation that the fungi, which produced the infected condition, invariably were found in this basal region.

An experiment was therefore carried out for the purpose of increasing the penetration of the sodium hypochlorite solution, and thereby increasing its effectiveness. Equal quantities of the disinfecting solution were added to two lots of 100 grains each. One of the flasks containing grains and solution was attached to a suction pump for one minute. This resulted in the rapid removal of considerable air in the form of bubbles from the grains. When the suction was released, the evacuated space was filled with solution. The other flask was not connected to the suction pump, and served as a control. Both flasks were shaken 2.5 hours.

The results of this experiment are shown in figure 4. It is evident that while the suction treatment reduced the germination over a 5-day period, as shown by the graph on the left, it also markedly reduced the number of infected grains. Of the 100 grains in each set, 9 grains of the control group became infected; only 1 grain was infected when the suction treatment was applied, as shown by the center graph. On the basis of 100 grains germinating with each treatment, 11.5 per cent. of the controls were infected,

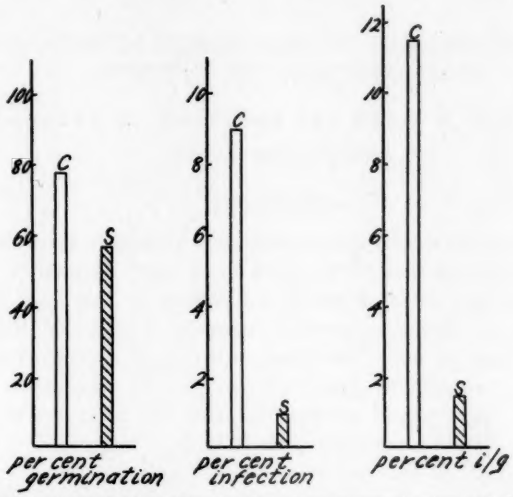

FIG. 4. Effect of suction injection treatment upon germination and infection of corn grains sterilized with sodium hypochlorite solution. Control (*C*) without suction treatment; grains (*S*) with suction treatment.

but only 1.75 per cent. of those exposed to suction treatment were infected, as shown by the graph at the right of the figure.

Summary

1. Increasing the length of time of treatment of corn grains with sodium hypochlorite solution from 0.5 to 5 hours reduced germination somewhat, whereas it greatly decreased the percentage of infected seed.

2. When sterilization with the disinfecting solution for an intermediate period of time was accompanied by a suction injection treatment, there was a marked reduction in the number of infected grains.

PURDUE UNIVERSITY
LAFAYETTE, INDIANA

LITERATURE CITED

1. DUGGAR, B. M., and DAVIS, ANNE W. Seed disinfection for pure culture work: the use of hypochlorites. Ann. Missouri Bot. Gard. **6**: 159–170. 1919.
2. ROBBINS, W. J. Cultivation of excised root tips and stem tips under sterile conditions. Bot. Gaz. **73**: 376–390. 1922.
3. TREADWELL, F. P., and HALL, W. T. Analytical chemistry. Vol. 2. p. 568. New York. 1928.
4. WILSON, J. K. Calcium hypochlorite as a seed sterilizer. Amer. Jour. Bot. **2**: 420–427. 1915.

VARIATIONS IN FLESHINESS OF TOMATO FRUITS AS AFFECTED BY FERTILIZATION[1]

KENNETH K. KRAUSOHE AND BASIL E. GILBERT

(WITH TWO FIGURES)

Introduction

The evaluation of quality in agricultural products affords many research problems. Primarily, there is the difficulty of defining quality factors and the worker does well to confine his efforts to those correlated with very concrete morphological or chemical differences which are easily measureable. The variations in quality which are noted by consumers may be caused by conditions existing during transportation or storage, or, they may have arisen from the cultural methods used by the producer. The variations in degrees of quality from production methods are not capable of extreme variation owing to the limits imposed by factors of heredity. With vegetables, however, some variations have been noted to occur from soil moisture and nutrient fluctuations.

A critical study of the response of certain market garden crops to fertilizer elements is being made on the experimental plats of the Rhode Island Agricultural Experiment Station. This study affords an excellent opportunity for research on the effect of the different elements on quality factors. During the summer of 1935, an attempt was made to determine whether a correlation might be found between the fertilizer ratios used and the amount of flesh found in the fruit of tomatoes growing on these plats.

Materials and methods

Pritchard tomatoes were grown on triplicate field plats, each $\frac{1}{63}$ of an acre in area. The fertilizers used in this experiment, their application in pounds per acre, and the fertilizer ratio are given in table I.

At intervals between August 12 and September 27, when the fruits were being harvested for market, samples were taken at random from all plats. They were then graded and only fruits of U. S. no. 1 grade were used.

A median cross section was made of each tomato. In order to insure as uniform a location as possible for the cut, a special gauge was constructed (fig. 1). This consisted of a platform which was capable of being raised or lowered and to which a carpenter's combination square was attached. The tomato was placed on the platform and the sliding arm of the square was

[1] Published by permission of the Director of Research as Contribution no. 475 of the Rhode Island Agricultural Experiment Station.

TABLE I

FERTILIZERS: THEIR APPLICATION IN POUNDS PER ACRE, AND RATIO

FERTILIZER	APPLICATION PER ACRE	FERTILIZER RATIO
Regular fertilizer	1500	5 –10–5
High nitrogen	1500	7.5–10–5
Low nitrogen	1500	2.5–10–5
High phosphorus	1500	5 –15–5
Low phosphorus	1500	5 – 5–5
High potash	1500	5 –10–7.5
Low potash	1500	5 –10–2.5
High chemicals	2000	5 –10–5
Low chemicals	1000	5 –10–5

adjusted until it rested on the blossom end of the tomato. A reading was taken of the length of the vertical axis of the fruit. The platform was then raised and clamped in position so that a knife resting on the two metal gibs (EE′, fig. 1) and sliding along them bisected the vertical axis of the tomato, cutting the fruit in half.

A piece of the tomato fruit was next laid with the cut face on a sheet of cardboard and its circumference was outlined in pencil. This outlined area, designated as the total area, was carefully cut out, numbered, and put aside for future measurement.

Using a special gauge (fig. 2 *H*), a slice one-eighth of an inch in thickness was cut from the half of the tomato.

The slice of tomato was placed on a metal screen, and, with the aid of a stream of water from a coarse nozzle, and tweezers, the seeds and their gelatinous matrix were removed. Carbon black was then sprinkled thickly over the slice to insure complete opacity.

To determine the areas of the slice of tomato and the cardboard disc, an area-measuring device similar to that described by BERGMAN[2] was constructed. This device consisted, in principle, of a light source and a lens which focuses the light on a glass screen on which the section of tomato was placed. The light passing this screen was collected by a second lens and focused on a Weston photronic cell which was connected with a millivoltmeter. The screen and light source were calibrated so that the effects of the light passing through the screen were known for the whole area, and any portion covered to prevent passage of light could be easily calculated from the decreased readings of the meter. The apparatus was calibrated by using discs of known area.

[2] BERGMAN, H. F. Unpublished report presented before American Society of Plant Physiologists, Boston, Massachusetts, December, 1933.

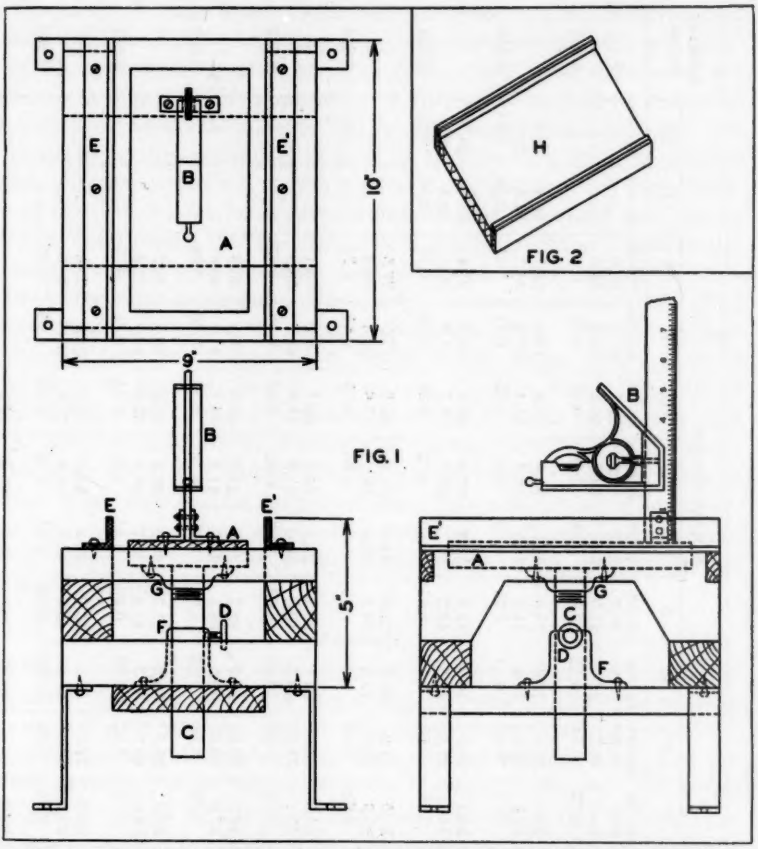

FIG. 1. Top, front, and side views of apparatus used in determining the position of slicing each tomato sample: A, platform; B, combination square; C, spindle; D, set screw; E and E', gibs; F, bushing flange; G, floor flange.

FIG. 2. Slicing gauge (H) used in determining the thickness of the tomato slice.

Discussion of experimental results

In table II are given the calculated areas, in square centimeters, of the tomato slices and cardboard discs. In order to relate these two areas the ratio used is as follows:

$$\frac{\text{sq. cm. of slice}}{\text{sq. cm. of disc}}$$

It will be noted that rather wide variations occur between individual samples of fruits. A consideration of the average ratios, however, shows

TABLE II

SHOWING AREAS IN SQ. CM. OF TOMATO SLICES AND CARDBOARD DISCS EQUIVALENT TO TOTAL AREA OF CUT TOMATO AND RATIO OF SLICE TO DISC IN EACH CASE

AREA AND RATIO	FERTILIZER TREATMENT	SAMPLE NUMBER												AVERAGE RATIO
		1	2	3	4	5	6	7	8	9	10	11	12	
Area of slice		*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*	
" " disc	Regular	24.4	30.3	25.9	27.8	20.3	21.2	42.2	32.1	21.3	20.0	.....	.....	
Ratio, slice/disc		36.8	40.3	40.7	38.7	32.0	36.6	63.9	50.2	33.6	33.6	.....	.....	
		0.66	0.75	0.64	0.72	0.63	0.58	0.66	0.64	0.63	0.59	.....	.....	0.651
Area of slice														
" " disc	High nitrogen	31.0	28.6	25.1	17.5	30.9	31.0	21.8	25.8	18.9	22.7	18.8	19.8	
Ratio, slice/disc		41.8	38.6	38.0	33.1	44.2	44.2	32.6	41.4	37.4	34.1	31.8	29.3	
		0.74	0.74	0.66	0.53	0.70	0.70	0.67	0.62	0.51	0.67	0.59	0.68	0.650
Area of slice														
" " disc	Low nitrogen	33.3	24.1	26.2	30.3	35.8	27.8	27.8	26.1	24.8	21.4	25.6	24.8	
Ratio, slice/disc		43.0	34.6	38.4	42.8	44.2	40.1	38.7	38.5	37.8	30.3	38.5	38.2	
		0.77	0.70	0.68	0.71	0.81	0.69	0.72	0.68	0.66	0.71	0.67	0.65	0.703
Area of slice														
" " disc	High phosphorus	37.7	21.5	21.9	24.0	23.6	34.9	24.8	25.6	22.2	27.5	.....	.....	
Ratio, slice/disc		48.7	35.9	30.3	43.4	36.1	49.4	38.1	33.6	36.4	38.8	.....	.....	
		0.77	0.60	0.72	0.55	0.65	0.71	0.65	0.76	0.61	0.71	.....	.....	0.674
Area of slice														
" " disc	Low phosphorus	32.2	30.5	33.5	24.9	25.4	24.9	24.5	26.1	30.0	26.1	.....	.....	
Ratio, slice/disc		50.5	45.1	51.6	40.7	40.4	30.0	37.6	38.4	42.7	39.9	.....	.....	
		0.64	0.68	0.65	0.61	0.63	0.83	0.65	0.68	0.70	0.65	.....	.....	0.672
Area of slice														
" " disc	High potash	33.0	34.2	28.9	25.7	22.7	21.2	27.5	19.9	28.2	34.5	.....	.....	
Ratio, slice/disc		46.3	47.5	45.5	41.0	36.1	35.0	41.8	34.1	47.1	49.8	.....	.....	
		0.71	0.72	0.64	0.63	0.63	0.61	0.66	0.58	0.60	0.69	.....	.....	0.646
Area of slice														
" " disc	Low potash	32.9	26.9	32.5	33.0	27.5	26.9	27.1	25.3	26.1	28.7	.....	.....	
Ratio, slice/disc		39.5	38.0	48.7	48.7	41.9	42.7	40.6	39.8	42.7	39.8	.....	.....	
		0.83	0.71	0.67	0.68	0.66	0.63	0.67	0.64	0.61	0.72	.....	.....	0.681
Area of slice														
" " disc	High chemicals	24.7	21.3	27.1	36.0	33.0	26.0	26.8	20.0	25.6	20.6	.....	.....	
Ratio, slice/disc		36.8	32.9	38.5	57.9	49.7	40.9	36.9	31.9	37.6	33.0	.....	.....	
		0.67	0.65	0.70	0.62	0.66	0.64	0.73	0.63	0.68	0.62	.....	.....	0.660
Area of slice														
" " disc	Low chemicals	29.2	28.2	26.0	22.2	20.8	26.6	25.2	22.3	.....	.....	.....	.....	
Ratio, slice/disc		43.6	40.2	38.3	35.8	35.2	39.3	40.5	33.6	.....	.....	.....	.....	
		0.67	0.70	0.68	0.62	0.59	0.68	0.62	0.66	.....	.....	.....	.....	0.653

that the group of fruits treated with high nitrogen fertilizer relatively had the least amount of flesh, while the fruits of plants treated with low nitrogen fertilizer had the greatest areas of flesh. That these differences are significant is shown by the application of STUDENT'S method of statistical analysis which gives odds of 96 to 1 to the difference between the high and low nitrogen fertilizer treatments, and 85 to 1 between the low and the medium nitrogen fertilizer of the regular chemical fertilizer treatment. None of the other comparisons gave significant odds. It would seem, therefore, from these results that the fleshiness of tomato fruits, and, conversely, the size of the cavities of the fruits may be related to the amount of nitrogen in the fertilizer treatments. The growing of tomatoes with little or no nitrogen tends to produce the greater amounts of flesh. While this may be desirable from the viewpoint of the consumer, visual observations of the firmness of the flesh lead to the opinion that the flesh of the fruits grown with the low nitrogen fertilizer treatment tends to be less firm and may be less desirable due to greater risk during marketing and storage. On the other hand, the fruits from the plants with the highest nitrogen fertilizer treatments had the largest cavities, thus possibly being less desirable for the consumer. It would seem therefore that a medium nitrogen fertilizer treatment would be the most desirable to insure good quality, as evaluated by fleshiness.

Summary

This study is concerned with the possible effect of fertilizers upon the fleshiness of tomato fruits. By measuring the area of the cross-sections of fruits with pulp removed, and by growing plants with varying fertilizer ratios, evidence was obtained to show that only the nitrogen content of the fertilizer gave significant differences in the degree of fleshiness. The fruits of the plants treated with the high nitrogen fertilizer tended to have relatively less flesh than the fruits of the plants treated with the low nitrogen fertilizer. The use of excessive amounts of nitrogen in the fertilizer may thus produce fruit with such large cavities that it injures the quality.

RHODE ISLAND AGRICULTURAL EXPERIMENT STATION
KINGSTON, RHODE ISLAND

BRIEF PAPERS

BEHAVIOR OF CITRUS FRUIT UNDER SPECIAL RESPIRATORY CONDITIONS AS AN EXPEDIENT INDEX OF VITALITY

(WITH THREE FIGURES)

There is an ever recurring demand for some simple indicator of the vitality of citrus fruit in relation to its keeping quality in storage and transportation. A few such indicators have been tentatively suggested, as for example the $KMnO_4$ method by KLOTZ.[1]

In April, 1935, the writers undertook to utilize toward this end the respiratory processes of citrus fruit under simple but specific conditions. The general method adopted consisted of filling with fruit a container capable of being made perfectly air-tight, and afterward recording the pressure changes as respiration of the fruit altered from approximately normal to the anaerobic type.

One kind of container used was of 2.5-gallon capacity and designed to withstand pressures of at least 50 lb. per square inch. These containers were equipped with mercury manometers 175 cm. in length. The chief characteristics observed in this set-up were the rate of change and the final pressure attained. The results proved very interesting but such containers were unwieldy and the end point was reached only after two to three weeks. The range of final positive pressures observed in this manner was 40 to 165 cm. of mercury. The curves of such pressure increase made, after an initial delay, a straight line day after day until near the end point. For convenience, and to shorten the period of observation, the method was modified to allow a closer following of pressure changes previous to reaching a positive pressure of 10 to 20 cm. of mercury. This condition called for batteries of low pressure containers consisting simply of 2-quart Mason jars of the "wide mouth" type, and finally, of 1-gallon wide mouth glass jars commonly used for mayonnaise (fig. 1). Both sizes of containers were equipped with mercury manometers 26 cm. long. The covers of the 1-gallon jars were made of steel disks 5 inches in diameter and $\frac{1}{4}$ inch thick. To these were cemented pure gum gaskets $\frac{1}{4}$ inch thick. Connections to the manometers were made by means of copper tubes passing through the center of the steel disks. The jars and lids were placed in strong oak frames so that the lids could be bolted down tightly while in use. The period of observation on fruit in these containers varied with the temperature. The majority of tests were made at 72° F., at which temperature the period was usually 24 to 72 hours. Many comparative tests, however, were carried out at 56°, 46°, 42°, and 38° F.

[1] KLOTZ, L. J. California Citrograph 18: 154–155. 1933.

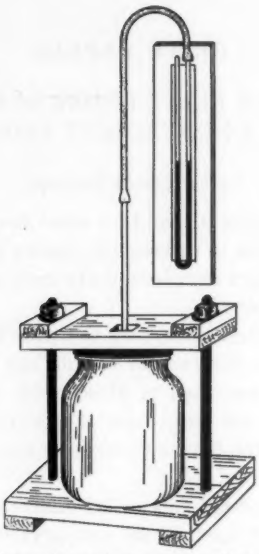

FIG. 1. Apparatus for determining index of vitality of fruits and vegetables.

A temperature of 56° proved satisfactory, except that the period of observation became about double that at 72°.

Fruit having an initial respiratory ratio of less than unity began to develop a negative pressure at once,[2] but after the development of nearly anaerobic conditions[3] the pressure began to rise, at first relatively slowly; with complete anaerobic conditions the pressure rose more rapidly and assumed a constant rate of increase throughout the remainder of the test.

Fruit having an initial respiratory ratio considerably greater than unity began to develop positive pressure at once; at first relatively slowly and falteringly, then more rapidly, from which time the rate of pressure increase became constant.

Apparently when the ratios were close to unity the early pressure changes were either slightly negative and then soon positive, or positive then slightly negative (or only dropping to a lower positive pressure) and finally strongly positive so that an "S" curve was shown when plotted. (See A and B, fig. 3).

The great variety of pressure changes produced by different fruits has been astonishing. The direction (positive or negative) and rate (slope of

[2] The relative values of the respiratory ratios mentioned here are assumed from fruit behavior in the tests.

[3] Chemical tests showed that the O_2 was almost completely absorbed by the time of maximum negative pressure.

the curve) of the pressure changes and the total negative pressure (if it occurs) seem to constitute the most valuable characteristics. When comparing curves constructed from data which show initial negative pressure two very good practical indices have been noted; namely, first, the number of hours elapsing before the curves return to the X-axis (*i.e.*, atmospheric pressure); and, second, the area of the figure bounded by the X-axis and that portion of the curve lying below it. The area was determined by means of a planimeter. Barometric changes are sometimes large enough to effect small variations in the curves.

Lemons have indicated that their respiratory ratio remains less than unity until a late stage of maturity, but the rate of development of negative pressure and return to atmospheric pressure decreases as the fruit matures. In respect to this characteristic, freshly picked lemons in general showed the following order, "dark greens"; "light greens"; "silvers"; and "tree ripe." However, silvers sometimes gave the greatest negativity, and frequently greater than dark greens. Typical curves of "dark green" and "tree ripe" lemons are shown in figure 2. The responses of such lemons after being held in storage for varying periods gradually became slower, although the relative position of the curves keep somewhat the same. After a few weeks at 56° F. the tree ripe lemons developed little or no negative

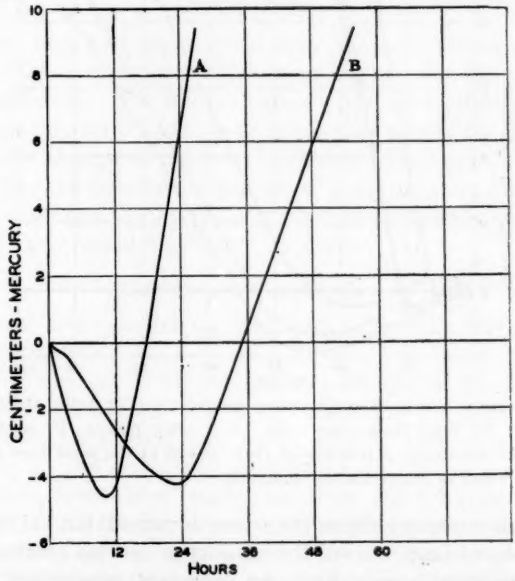

FIG. 2. Pressure curves of freshly picked lemons. Test made at 72° F. A, "dark green"; B, "tree ripe."

pressure, at which stage they had probably almost passed the period of commercial usefulness. Extensive tests are under way for correlating the characteristics of the pressure curves obtained from lemons with the subsequent behavior of the latter in transit and market. The present respiratory tests on lemons have shown in addition to the above, effects of oil spraying, relative humidity, temperature, irrigation, and ethylene treatments. Some effects of ethylene seemed still to be in evidence nine weeks after treatment.

Immature grapefruit (of pre-harvest season) have indicated respiratory ratios less than unity, but grapefruit fresh from the grove at early commercial harvest season have shown ratios slightly greater than unity. The pressure curves of the latter type were usually "S" shaped (fig. 3). The

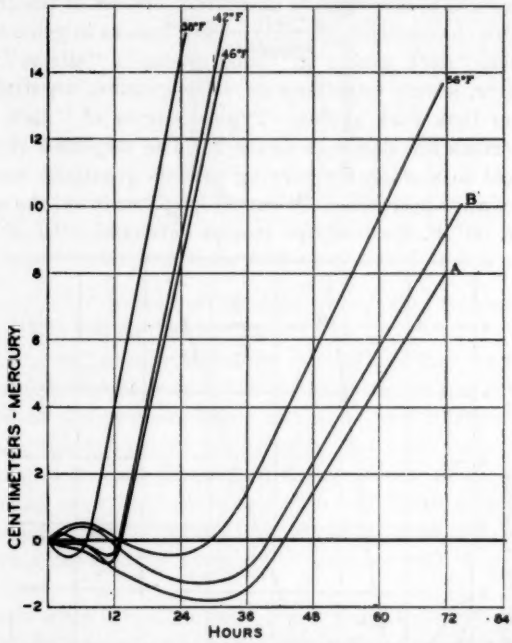

FIG. 3. Pressure curves of early season grapefruit (Coachella, California). Tests made at 72° F. All fruit from same trees. A, freshly picked; B, as A but picked 6 weeks later. The remaining curves are of fruit picked at the same time as A, but held in storage for 6 weeks at temperatures indicated.

ratios did not increase rapidly as the season advanced, but old fruit from the tree, or long-stored fruit, showed the capacity to develop continuous positive pressures indicating large ratios. An increased respiratory activity has been noted for grapefruit which have pitted in storage.

A relatively small number of tests have been made upon late season Valencias (1935) and the present season (1935–36) Washington Navel oranges. The results indicate a situation closely resembling that for grapefruit with perhaps a slightly greater tendency to show initially positive curves.

In a cursory manner the special respiratory test has been made upon apples, pears, grapes, tomatoes, lettuce, and other fruits and vegetables. The responses were frequently very interesting and suggested that the method might have a wider application than to citrus, as a simple and rapid test of maturity or vitality.

The work of correlating the types of pressure curves obtained from fruit with its likely subsequent behavior on the market is very great. The practical value of the method can be determined only through its trial by many workers upon different varieties of fruit and in different localities. This situation is taken as justification for this preliminary report.—E. M. Harvey and G. L. Rygg, *Bureau of Plant Industry, U. S. Department of Agriculture, Pomona, California.*

BEHAVIOR OF STOMATA OF IRRIGATED WHEAT PLANTS[1]

Introduction

It is known that stomata respond very markedly to environmental changes, especially to variations in the water supply, but their reaction is often very complicated. The writers have studied the stomatal movements of irrigated wheat plants in order to determine the possibility of using certain characteristic changes as indices of different irrigation procedures, and especially for the determination of the time of acute necessity for irrigation.

The work was carried on during the summer of 1933 in the Volga district at the Valuika Agricultural Experiment Station.

Materials and methods

The soft wheat, *Erythrospermum* 0841, sown on April 26, was the material used in our investigation.

The degree of opening of the stomata was determined by the alcoholic method of Lloyd. Counts were made, with a microscope, of the number of stomata that were fully open, half open, slightly open, and completely closed. A fully opened stoma was about twice as large as one half open, and these, in turn, three times larger than those slightly open. The total opening for all the stomata was calculated by multiplying the percentage of fully opened stomata by 10, the half opened by 5, and the slightly opened by 1.6. Thus, if all the stomata were completely open, the greatest number

[1] A more detailed account of the results obtained in this investigation will be presented in another paper.

obtained would be 1000. Counts of the stomata were made seven times during the day: at 5, 7, 9, and 12 A.M., and 2, 4, and 6 P.M.

Investigation

Observations on stomatal behavior

A record was made of the weather, the amount of moisture applied to the wheat plants, and their stage of development. The following observations were made of the effect of these factors on the degree of stomatal opening on June 16, 22, and 30.

June 16.—The day was cloudy. The soil (about 60 cm. deep) under the plants that were irrigated on June 1 still contained 2 to 3 per cent. available water; the soil under the control plants was so dry that it almost reached the wilting coefficient. The plants were at the beginning of head formation. Table I gives the calculated amount of stomatal opening at various hours during the day, and the average for the day.

TABLE I

Condition of stomata on June 16

Irrigation	Calculated amount of stomatal opening							
	5 A.M.	7 A.M.	9 A.M.	12 M.	2 P.M.	4 P.M.	6 P.M.	Av. for day
Non-irrigated (control)	177	368	179	157	186	17	157	177
Irrigated on June 1	898	844	967	711	850	698	728	814

The stomata of the irrigated plants were open the entire day on June 16; the stomata of the non-irrigated plants opened for a short time early in the morning and were opened but very little during the remainder of the day.

June 22.—The early morning of June 22 was cloudy, but the weather was clear after 9 A.M. There was a 0.7 mm. rainfall on the day preceding which slightly moistened the upper layer of soil. Two groups of plants were studied: one group which was irrigated on June 1, and the other irrigated on June 1 and 19. The plants were in bloom when the observations on stomatal behavior were made (table II).

The small amount of rainfall on the day preceding June 22 seemed to cause the control plants to open their stomata rather wide in the forenoon, but in the afternoon the stomatal apertures became narrower.

The behavior of the stomata in the group of plants irrigated on June 1, which, according to determinations of the soil moisture, had exhausted nearly all of the water supplied, was different from that of the non-irrigated control plants. Their stomata were closed even earlier in the day than

TABLE II

CONDITION OF STOMATA ON JUNE 22

IRRIGATION	CALCULATED AMOUNT OF STOMATAL OPENING							
	5 A.M.	7 A.M.	9 A.M.	12 M.	2 P.M.	4 P.M.	6 P.M.	AV. FOR DAY
Non-irrigated (control)	166	381	897	771	256	.....	262	456
Irrigated on June 1	196	785	911	305	367	407	251	460
Irrigated on June 1 and 19	908	979	933	906	972	908	893	928

those of the control plants. This was possibly due to the greater development of their transpiring surface.

The group of plants which were irrigated on June 1 and 19 had 10 per cent. available moisture remaining in the soil. Their stomata were uniformly open throughout the day.

JUNE 30.—The entire day of June 30 was cloudy. A rainfall the preceding day moistened the soil very slightly. Observations made on the behavior of the stomata on this day are given in table III.

TABLE III

CONDITION OF STOMATA ON JUNE 30

IRRIGATION	CALCULATED AMOUNT OF STOMATAL OPENING							
	5 A.M.	7 A.M.	9 A.M.	12 M.	2 P.M.	4 P.M.	6 P.M.	AV. FOR DAY
Non-irrigated (control)	175	266	250	225	177	173	8	183
Irrigated on June 1	953	888	782	964	815	397	116	702
Irrigated on June 1 and 19	972	925	967	905	844	978	935	932

Regardless of the slight rainfall, the non-irrigated control plants had their stomata open but little during the day, and they were closed about 6 P.M. The plants of the group irrigated on June 1, because of the cloudy weather, were still disposing of the remainder of the soil moisture. Their stomata remained open until 12 A.M. After 2 P.M. they gradually began to close. Plants irrigated on June 1 and 19, and therefore having a good supply of water, had their stomata almost fully open during the entire day. Further observations on the plants were discontinued because of a serious attack of rust.

A study of the results of the observations shows that there was a marked difference in the behavior of the stomata of plants grown with and without irrigation, and of the stomata of those grown with various amounts of irrigation. Stomata of non-irrigated plants opened but little, and only early in the morning, while the stomata of plants with a sufficient water supply were fully open throughout the day.

Effects of stomatal behavior

The differences in stomatal behavior between the non-irrigated and irrigated plants seem to have affected their assimilation. This was concluded from a comparative study of the recorded dry weights, and yields of grain, of the plants at maturity. The comparative dry weight of 100 plants in the various groups at maturity was as follows: non-irrigated (control) plants, 171 gm.; plants irrigated on June 1,—271 gm.; plants irrigated on June 1 and 19,—358 gm. The comparative yield of grain from each of these three groups was 69, 109, and 130, respectively.

Summary

These experiments on stomatal behavior, in general, show that the degree of opening of the stomata during the day may serve as an index of the amount of water available to the plant. From this study the writers believe that valuable data may be obtained for working out a definite schedule for the irrigation of wheat.—N. A. Maximov and Lydia K. Zernova, *Research Institute of Grain Farming, Saratov, U. S. S. R.*

TOXICITY OF MERCURY VAPOR TO GERMINATING TOBACCO SEEDS

Injury to plants caused by mercury vapor has been reported by several investigators, but the danger of accidental poisoning of laboratory material does not seem to be generally recognized. This paper presents a study of toxicity in relation to the area of mercury exposed, the volume of inclosed space remaining constant.

Zimmerman and Crocker (**4**) studied the injury by mercury vapor to plants of about 75 genera, tobacco plants being among the few which were found to be comparatively resistant. The degree of injury depended on the amount of metallic surface exposed to the air. Kipp (**3**) observed an inhibiting effect of mercury from a mercury vapor pump on the respiration of tobacco seeds, but, when the seeds were removed from the apparatus after 66 hours, their viability was not impaired. It was found necessary to introduce a trap between the pump and the germinator in order to avoid this toxic effect.

The writer became interested in this problem when tobacco seeds failed to germinate under conditions which indicated that they had been poisoned by mercury vapor. A glass culture vessel was used as a moist chamber to enclose Petri-dish germinators, in which the seeds were sown on moist filter paper. The moist chamber became contaminated with liquid mercury, and in the next three trials the seeds failed to germinate during 8 or 10 days in the moist chamber. They germinated very poorly during subsequent incubation outside the moist chamber, indicating that their viability had been seriously impaired. After the vessel had been washed once with concentrated nitric acid, 51 per cent. of the seeds germinated, but the seedlings were stunted. After a second washing, excellent germination and normal seedlings were obtained, indicating that the toxic material had been removed.

Experiments were conducted to determine whether toxicity, as indicated by reduction in percentage of germination and stunting of tobacco seedlings, varied directly with the area of mercury exposed, the volume of the inclosed space and the temperature remaining constant. The moist chambers consisted of 4-liter battery jars, containing 100 cc. of distilled water and a galvanized iron wire support for the dishes, and covered with a glass plate. In each moist chamber there were three Petri dishes. Each dish contained two sheets of filter paper moistened with distilled water, on which were sown 100 seeds of no. 301, a cigar-wrapper variety. A glass vial, containing enough mercury to cover the bottom, was placed in each jar on the top of the stack of dishes. The vials were 7 cm. deep, and from 0.3 to 4.6

TABLE I

GERMINATION OF TOBACCO SEEDS INCUBATED FOR 14 DAYS IN 4-LITER BATTERY JARS CONTAINING VIALS OF MERCURY, AND LATER FOR 14 DAYS IN JARS CONTAINING NO MERCURY. VALUES GIVEN ARE AVERAGES OF RESULTS OF TRIPLICATE DETERMINATIONS

VIAL OF MERCURY		GERMINATION OF SEEDS IN FOUR TRIALS									
		WITH Hg					LATER WITHOUT Hg				
DIAM.	AREA*	1	2	3	4	Av.	1	2	3	4	Av.
cm.	*sq. cm.*	%	%	%	%	%	%	%	%	%	%
.....	.....	84	78	85	82	82	84	82	85	84	84
0.3	0.07	.....	68	81	73	74	.....	80	85	80	82
0.5	0.20	24	68	52	49	48†	52	78	77	61	67†
0.7	0.38	0	.....	.....	.....	0	4	.....	.....	.....	4†
1.3	1.33	0	0	0	0	0	6	1	12	23	11†
4.6	16.62	.....	0	0	.....	0	.....	0	3	.....	1†

* Cross-sectional area of the vial.

† Seedlings stunted.

cm. in inside diameter. Controls received no mercury. The seeds were incubated in light at a temperature of 24° ± 1° C., the optimum for these seeds (**2**). After 14 days, the seedlings were counted and removed from the Petri dishes. Then the seeds remaining ungerminated were incubated for 14 days more in moist chambers containing no mercury, and a final count was made. The averages of triplicate determinations in four consecutive trials are given in table I.

These results indicate that toxicity varies directly with the area of mercury exposed, and that the area of mercury to which germinating tobacco seeds may be exposed under these conditions without apparent injury is less than 0.07 sq. cm. in 4 liters of inclosed space, or 0.018 sq. cm. per liter.

The toxicity of mercury under these conditions should vary directly with the temperature, as does the vapor pressure of liquid mercury (**4**). This hypothesis is supported by the results of some preliminary tests conducted at room temperature, ranging from 15° to 25° C. At this lower average temperature, toxicity was much less than at 24° C., as shown by the fact that there was practically no injury with 1.33 sq. cm. of mercury in 4 liters, or 0.33 sq. cm. per liter.

These observations suggest that caution must be exercised in the use of mercury devices, such as pumps, manometers, and seals in biological apparatus. ZIMMERMAN and CROCKER (**4**) reported that a thin film of water over the surface of the mercury was sufficient to prevent a toxic effect on plants. DAFERT (**1**) believed that glycerin was more efficient in this respect than either water or mineral oils.—RANDALL R. KINCAID, *North Florida Experiment Station, Quincy, Florida.*

LITERATURE CITED

1. DAFERT, J. W. Poisonous action of mercury on green plants. Zeitschr. Landw. Versuchsw. Oesterr. **4**: 1–10. 1901. (Abs. in Exp. Sta. Rec. **13**: 716. 1901–1902.)
2. KINCAID, RANDALL R. The effects of certain environmental factors on the germination of Florida cigar-wrapper tobacco seeds. Florida Agr. Exp. Sta. Tech. Bull. 277. 1935.
3. KIPP, MARGARETE. Die Abgabe von Kohlensäure und die Aufnahme von Sauerstoff bei der Keimung lichtgefördeter Samen von *Nicotiana tabacum*. Jahrb. wiss. Bot. **71**: 533–595. 1929. (See pp. 582–583.)
4. ZIMMERMAN, P. W., and CROCKER, WILLIAM. Plant injury caused by vapors of mercury and compounds of mercury. Contrib. Boyce Thompson Inst. **6**: 167–187. 1934.

MODIFICATION OF ECKERSON METHOD FOR DETERMINING NITRATE REDUCASE[1]

A satisfactory method for measuring the nitrate reduction by plant extracts should throw some light upon the ability of the plant to assimilate nitrogen in this form. A method for determining the nitrate reducing activity of plant extracts was worked out by ECKERSON.[2] A brief statement of her method follows. Extracts of plant tissues are secured by grinding the fresh plant material very finely in a food chopper, and pressing out the juice through cheesecloth. One cubic centimeter of this juice and 1 cc. of 10 per cent. potassium nitrate solution containing 10 mg. of freshly dissolved glucose are made up to 10 cc. with water. This mixture is brought to a pH of 7.2 to 7.4 with sodium hydroxide, using phenol red as an indicator. In the original papers it was contended that there were enough natural buffers present to resist any great changes in hydrogen ion concentration; but, on the other hand, it was pointed out that there was a gradual increase in hydrogen ion concentration during the incubation period. The mixture is incubated under toluene at 35° C. for 17 hours. After incubation the amount of nitrite present is determined by the sulphanilic acid method.

The writer and his coworkers found that, although the solutions used in the ECKERSON method were adjusted to the same pH at the beginning of the incubation period in the manner just described, there was an unequal decrease in pH, so that at the end of the period the extracts from different sources showed various degrees of acidity. Some of these extracts probably contained a greater quantity of a natural buffer. The change in pH can be prevented by substituting 4 cc. of 0.2 M phosphate buffer mixture (pH 7.3) for one-half of the water used in making up the original volume.

The coloring matter in most plant tissues interferes with a comparison of the color produced by the nitrite reagent. It was found that by removing these colored substances with activated charcoal before treating with the sulphanilic acid reagent, a satisfactory comparison could be made. Upon being removed from the incubator the solutions are treated with 5 gm. of activated charcoal. After being thoroughly mixed and allowed to stand for 15 minutes they are washed through a fine filter paper into 100-cc. volumetric flasks. The flasks are made up to volume and 10-cc. aliquots are used in the determinations. None of the nitrite is absorbed. A standard solution of nitrite treated in the same manner was not affected by the charcoal, since the entire amount of nitrite could be recovered in the filtrate. For solutions which are difficult to clear with charcoal, a second clearing with 2 gm. of

[1] Contribution from the Department of Horticulture, Missouri Agricultural Experiment Station. Journal Series no. 461.

[2] ECKERSON, S. H. Influence of phosphorus deficiency on metabolism of the tomato. Contrib. Boyce Thompson Inst. 3: 197–218. 1931.

very fine magnesium oxide is generally effective. For most solutions the use of the magnesium oxide is unnecessary. Solutions for colorimetric comparison with a standard should be colorless and perfectly clear. These solutions keep fairly well for several days but there is a gradual loss of nitrite.

This method is fundamentally the same as the one first described by ECKERSON. The modifications suggested simplify the determination, and make it more adaptable to varying materials and conditions.—A. D. HIBBARD, *College of Agriculture, University of Missouri.*

NOTES

Annual Election.—The secretary-treasurer, Dr. W. F. LOEHWING, has announced the results of the various elections, including the annual election of officers, as follows: President, Dr. R. B. HARVEY, University of Minnesota; vice-president, Dr. OTIS F. CURTIS, Cornell University. Elective members of the executive committee were chosen as follows: Dr. D. R. HOAGLAND, University of California, (1939); Dr. H. R. KRAYBILL, Purdue University, (1938); Dr. JOHN W. SHIVE, Rutgers University, (1937). The editorial board has been enlarged by election of three members, and modified by appointments, as follows: Dr. BURTON E. LIVINGSTON, Johns Hopkins University, serving an appointive term on the editorial board, was elected to a three-year term (1939); to fill out his unexpired appointive term the executive committee appointed Dr. WILLIAM H. CHANDLER, University of California (1938). Dr. EDWIN C. MILLER, Kansas State Agricultural College, was elected to a two-year term (1938); and Dr. OTIS F. CURTIS, Cornell University, to a one-year term on the board (1937). At the St. Louis meeting, Dr. MAX A. MCCALL, U. S. Department of Agriculture, was appointed to a five-year term on the editorial board to replace Dr. C. R. BALL, U. S. Department of Agriculture, who had given distinguished service in this capacity for ten years. The newly elected officers are listed on cover page three of this number of PLANT PHYSIOLOGY, and the newly constituted editorial board on cover page two.

Rochester Meeting.—The summer meeting of the American Society of Plant Physiologists was held at Rochester, New York, on June 17, and at Geneva, and Ithaca, New York, on June 18, 1936. The program on June 17 was a joint program with the Physiological Section of the Botanical Society of America, and with the American Society for Horticultural Science. This single joint program was so satisfactory, and so well attended, that it has set a high mark of excellence in the way of summer programs. The attendance fluctuated from 100 to 120 throughout the day. On Thursday June 18, the inspection trips through the State Experiment Station at Geneva, and the Cornell Station at Ithaca, were followed by a large and enthusiastic group, with sustained interest through the day. With this experience with simplified programs a matter of history, it should be easier for program committees to have courage to maintain a similar simplicity in future meetings. The Rochester, Geneva, Ithaca meeting was decidedly popular and satisfying. Much credit for the success of the meeting goes to Dr. H. C. THOMPSON, of Cornell, and to Dr. D. R. GODDARD, of the University of Rochester, whose capable services were greatly appreciated.

The executive committee held a single session, but one of the most significant sessions in the history of the society. The STEPHEN HALES endowment

fund was enlarged to meet the current trends in return on investments, and provision was made for growth of the general endowment. The wisdom of this action will not be impressive until time has elapsed, but in later years it is likely to be looked upon as an act of rare foresight.

In addition, the executive committee decided that the railway fare of the president of the society to the regular meetings should be paid, and that in case he is unable to attend, this courtesy shall be extended to the vice-president. All of these actions involving financial arrangements are justified by the fundamentally sound financial condition of the treasury.

Other actions taken were of great importance. Some of these may be reported at a later time, when the information is more vitally needed.

Atlantic City Meeting.—In common with the other biological societies, the A.S.P.P. will be meeting with the A.A.A.S. at Atlantic City in December, 1936. This is one of the quadrennial meetings of the A.A.A.S., and there should be a large attendance. Early arrangement of everything pertaining to the meeting is desirable, with special attention to the convenience of the arrangements. With our membership now so large, it is more necessary to plan carefully, to prevent overcrowding of programs, and to find rooms adequate to our needs. Cooperation of the entire membership on these problems is desirable. With the progressive leadership of the program committee, which is headed by Dr. F. P. CULLINAN, chairman, with Dr. ANNIE M. HURD-KARRER and Dr. JOHN W. SHIVE as aids, this meeting may be made one of outstanding value.

Minnesota Section.—The Minnesota Section has held a number of meetings during the year. The high spot of the year's activities centered around the celebration held in honor of the Russian physiologist, PAVLOV. This celebration, of which the Minnesota Section was a sponsor, was attended by several hundred people representing all departments of science, and the general public. Other meetings gave opportunity for those who had been abroad to report on their observations in foreign laboratories. Dr. SHIRLEY at one of the meetings discussed the forest experiment stations, and seed laboratories of Germany and Switzerland, and Dr. BURR at another meeting gave an account of foreign laboratories of plant physiology which he had visited while in Europe. Mrs. ABBE presented a paper on the physiological functions of phloem structures, and the relation of these to the finer structures which are revealed by microchemical studies. Most of the meetings are held at the University Farm Union, with 25 to 30 in attendance.

New England Section.—The annual meeting of the New England section was held at Connecticut State College, Storrs, Connecticut, May 8 and

9, 1936. The program of Friday afternoon, May 8, was devoted to papers, and the attendance was about 60. A symposium on light and light measurements Saturday forenoon, May 9, attracted an attendance of over 100. The nature of this program is indicated by the six general topics included: A general survey of the radiation spectrum and distribution of energy within it, by Dr. B. E. GILBERT; a survey of selective phenomena, as by the human eye, by plants in photosynthesis and phototropism, and by photochemical processes, by Dr. T. G. PHILLIPS; pyrheliometers, their types, characteristics, and usefulness, by Dr. P. R. GAST; photocells, types, characteristics, dependability, permanence, and selectivity, by JOHN L. REINARTZ; atmospheric absorption of radiation, smoke, water vapor, haze, fog, clouds, daily and seasonal angle of incidence, etc., by Dr. C. G. DEUBER; sampling of radiation, flat horizontal surface, single flat surface in one direction, multiple flat surfaces in various directions, by Dr. R. H. WALLACE.

Summarized from a round table discussion, the symposium brought out the following facts: "There is sufficient evidence to state that the effect of light on plant physiological problems is such that the measurements should be made of the visible spectrum plus the extreme red and ultraviolet. The possible influence of the infra red rays could not be denied entirely, in view of their direct or indirect action in increasing the dry weight of some plants when added to the visible spectrum. Measuring radiation was deemed important in connection with any problems of physiology involving receipt of radiant energy by the plant. Detailed discussion involved the respective merits of various photronic cells, and pyhreliometers, mounted in either horizontal or equatorial positions. Finally it was demonstrated clearly that all measurements of radiant energy should be correlated and checked carefully with the use which plants make of the energy they receive. The possibility of using some plant process as a measure of energy reception and utilization was held to be a desirable goal for future work, in conjunction with efforts at better standardization of instruments and sampling technique."

Space does not permit mention of all of the fine social arrangements, which included a banquet held in a quaint old New England church, the Spring Hill Church, on Friday evening.

The new officers of the section are Dr. B. E. GILBERT, chairman; and Dr. F. H. STEINMETZ, vice chairman. Dr. JONES remains secretary-treasurer. The next meeting will be held at Kingston, Rhode Island, in 1937.

We are happy to announce that mimeographed copies of the subject matter in the light symposium are available at 15 cents per copy. Send money with order to Dr. LINUS H. JONES, Clark Hall, Massachusetts State College, Amherst, Massachusetts.

Western Section.—The Western Section of A.S.P.P. held its first annual meeting at Seattle, on June 17 and 18. On Wednesday the meeting was a joint one with the Western Society of Soil Science at which eleven papers were read, with attendance between 35 and 75 during the day. At a business meeting of the section, a constitution was adopted, and the section was affiliated with the Pacific Division of the A.A.A.S. The program of Thursday, June 18, was devoted to papers, ten of which were read before the section. New officers will be chosen later in an election to be held after the pattern of elections in A.S.P.P. The new section has made a good beginning, and is now ready to enjoy all of the advantages that go along with regional cooperation. Its meeting places will be with the Pacific Division, A.A.A.S.

Purdue Section.—The Purdue Section carried out its customary program during the year by holding semimonthly afternoon meetings which were addressed by staff members. The opening and closing of the University year were celebrated by dinner meetings at which the ladies were entertained. Dean emeritus STANLEY COULTER addressed the opening meeting in the fall on "Conservation in Indiana." Dr. NEIL STEVENS of the University of Illinois was the spring meeting guest, whose subject was "An outline of scientists."

For the year 1936–1937, R. B. WITHROW has been elected chairman of the section, and D. M. DOTY, secretary.

Annual Review of Biochemistry.—The appearance of a new volume of the *Annual Review of Biochemistry* has become an event of importance among biochemists and physiologists. Volume V, which has recently come from the Stanford University Press, is a worthy successor to the preceding fine volumes in this annual series. It will make a very favorable impression among those who most fully appreciate the value of the series as a whole from frequent use. The 26 reviews cover a large segment of the field, and the subjects covered will make an unusually wide appeal to physiologists who find that biochemistry is the life blood of physiological science. Among the reviews most valuable to plant physiologists may be mentioned QUASTEL'S review of enzymes; SPONSLER and DORE'S x-ray studies of the structure of compounds of biochemical interest; HAWORTH and HIRST'S chemistry of the carbohydrates and glucosides; HART and ELVEHJEM'S mineral metabolism; RUHLAND and WOLF'S metabolism of carbohydrates and organic acids in plants; NIGHTINGALE'S biochemistry of the nitrogenous constituents of the green plants; MAZÉ'S rôle of special elements in plant nutrition; KLUYVER'S bacterial metabolism; WAKSMAN'S soil microbiology; and IWANOFF and ZWETKOFF'S biochemistry of fungi.

In addition to these, there are reviews covering biological oxidations and reductions, hormones, water-soluble vitamins, fat metabolism, metabolism of amino acids, carbohydrate metabolism, the chemistry and metabolism of sulphur compounds, and of phosphorus compounds. Every section will prove valuable to those who maintain an interest in general physiology.

The reviews maintain the high standards set for the series. The material has been selected with skill and judgment, and the direction of progress is delineated as clearly as is possible with the mass of material to be examined each year. One seldom obtains so much valuable information for a $5.00 investment. The libraries of all active plant physiologists need this annual summary of biochemical advance. Orders for it may be sent to the Stanford University Press, Stanford University, California.

Experimental Enzyme Chemistry.—A very helpful volume with this title has been written by Dr. HENRY TAUBER, of the New York Homeopathic Medical College and Flower Hospital, New York City. It is published in mimeoprint form, with flexible binding, by the Burgess Publishing Co., Minneapolis, Minnesota. The work summarizes briefly (it contains but 118 pages) the present experimental status of enzyme chemistry. The first chapter is general, with brief statements concerning temperature effects, pH, specificity, mass action, various equations and theories, kinetics, activators and inhibitors, mechanism of enzyme reactions, preparation of enzyme materials, etc.

There are ten chapters devoted to groups of enzymes: esterases, proteolytic enzymes and peptidases, amidases, carbohydrases, catalase, oxidizing enzymes, the flavine oxidation system of WARBURG, carbonic anhydrase, the zymase complex and alcoholic fermentation, and luciferase. Extensive bibliographies are included at the close of each chapter. The information is condensed, but should prove very valuable and stimulative to investigators in the enzyme field, or to those who wish to extend their acquaintance with experimental technique of enzyme chemistry. The quoted price is $3.50 per copy; orders should be addressed to the publishers at 426 South Sixth St., Minneapolis.

Biochemistry of the Lipids.—Professor HENRY B. BULL, of the biochemistry department of the University of Minnesota, is the author of a monograph on the biochemistry of the lipids, which also is issued in mimeoprint form by the Burgess Publishing Co. The account is highly condensed, and covers a large field in a small space. It contains only 136 pages, but it is brimming with valuable information. The general nature of the presentation may be indicated by the main section headings. Following a very brief introduction, the following subjects are considered: fatty acids, soaps, alcohols, hydro-

carbons, sterols, fats and oils, phospholipids, glycolipids, emulsions, carbohydrate esters of fatty acids, and the physiology of the lipids in both animals and plants. As monographic information on the lipids is not plentiful, and as some of the older works are now too old to be very useful, this summary by Dr. BULL should find an appreciative demand from plant physiologists. The lipid field is one that deserves much more attention from physiologists than it has received.

The quoted price of this useful volume is $3.25 per copy. Orders for it may be sent directly to the publishers.